The Wildlife-Livestock Interface of Infectious Disease Dynamics:
A One Health Approach

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy
in the Graduate School of The Ohio State University

By

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2016

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Abstract

Surveillance for wildlife diseases is critical to our understanding of the emergence, transmission, persistence and control of infectious diseases at the interface of humans, domestic animals, and wildlife populations. *Neospora caninum* is a protozoan parasite capable of infecting a wide range of canid and ungulate species. The importance of the disease relates to economic losses, mainly derived from endemic or epidemic abortions in cattle. In the United States, coyotes and dogs are believed to be the main definitive hosts and white-tailed deer and cows are the main intermediate hosts. Our overall aim was to better understand the wildlife-livestock interface of *N. caninum* in natural settings. First, we estimated the true prevalence of *N. caninum* in three ruminant species by using Bayesian inference. We identified and discussed differences between apparent and true prevalence (TP). Differences in TP for some species suggest differences in the epidemiology of *N. caninum* for these co-located populations. Second, we evaluated the environmental phase of *N. caninum* shed in wild canid scats. Results suggested that the role of this environmental phase in the transmission to ruminants is likely minor. Finally, we evaluated the role of host species heterogeneity in the epidemiology of *N. caninum* circulating in a community. We identified differences in the patterns of immunity, age structure, and maternal and/or fetal antibody duration in three intermediate (ruminant) host species. Also, we estimated the species-specific
contributions to the persistence of this pathogen in a community. This research was approached from the One Health perspective and provided a better understanding of *N. caninum* dynamics at the wildlife-livestock interface in an ecosystem.
Dedicated to my mother, Maria de los Angeles Torres Castellanos, my sisters, Lorena and Itzel, my brother, Jesus, and my significant other, David A. Ramirez Cadavid, for their love and infinite encouragement in each step in the journey of my life.
Acknowledgments

I wish to thank my advisors for helping me to succeed and rise to a new peak in my career. To Rebecca Garabed, for giving me the opportunity to pursue this dream, for supporting my independency and for empowering my ideas. To Mark Moritz, for opening my mind to a new perspective on human dimensions, for guiding me in stressful times and for your clear and organized thoughts. To Barbara Wolfe, for transmitting to me your passion for wildlife and for your wonderful support on network building, such as opening collaboration with the Wilds, that made this project possible. To William Saville, for your enthusiasm and encouragement to make me a better scientist and for your effort to obtain funding that allowed me to successfully complete chapter 3.

I would sincerely like to thank Laura Pomeroy for her endless support as a colleague and as a friend. Her countless hours of answering me and philosophizing with me about infectious disease modeling made pleasant and fruitful my journey.

In addition, I want to take this opportunity to recognize all undergraduate and graduate students for volunteering and helping me to keep up with this dream: Brad Ryan, Pallavi Oruganti, Erika Helgeson, Devinn Sinnott, Katy Weaver, Laura Faris, Jeaniene Leis, Emily Morehouse, Katherine Rossos, Darcy Doran-Myers, Jessie Scaglione, Adam Jacin, Hannah Ghindea and Paulynne Bellen, and the list can keep going… THANK YOU!
Many thanks to the farmers, hunters, The Wilds’ and Muskingum Livestock Auction’s staff, for believing in and making possible this project.

To my friends for their endless support, laughs and encouragement: Natalia Higuita, Daniel Gallego, Anusha Manava, Maria Elena Hernandez, Hyeyoung Kim, Andrea Cordoba, Adrian Barragan, Lohendy Munoz, Mohamed El-Gazzar. My dear volleyball forever friends Jacki, Ale, Glo, Sara, Vane, and my coach Sergio. Finally, thanks to Maria Fernanda Salazar, Claudia Olguin and Silvia Ruiz, for always supporting my battles.

To my mother Angeles, I want to thank you for encouraging me and supporting me in all aspects of my live, including my passion for science (Te quiero mucho mamá!).

To my nephew, Derek Marichal Moreno, for remind me that life is about laughing, hugging and playing.

It goes without saying thanks to my father, Jesus Moreno, for teaching me that the real world is not made by super heroes, but by people that make decisions.
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Fields of Study

Major Field: Comparative and Veterinary Medicine

Studies in Epidemiology, Disease Ecology, and Infectious Disease Modeling
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Chapter 1: A One Health Approach for Multi-host Pathogens:

The case of *Neospora caninum*

Understanding multi-host infectious disease systems is one of the major challenges to scientists, public and animal health practitioners, and policy makers in the 21st century [1]. Multi-host pathogens are characterized for their complex life cycles, involving a wide range of hosts—human, wildlife and domestic populations—at each of their infectious stages [2]. In addition, multi-host pathogens may regulate hosts’ populations and communities, including predator, prey and competitor host species [3]. Coupled with this is the diversity of research at various scales from within-host dynamics to ecosystem level processes, and the combination of theoretical, observational and experimental approaches to elucidate strengths and weaknesses of those systems [4].

The importance, from the human point of view, of elucidating strengths and weaknesses of multi-host pathogen systems is to diminish the threat to human health, economic burden and loss of biodiversity [5]. For example, global trends in emerging infectious diseases in humans showed that 60.3% of emerging infectious disease events are caused by zoonotic pathogens, defined as those that are non-human animal source, and from those 71.8% are caused by wildlife pathogens [6]. Additionally, there are many other multi-host diseases that are not zoonotic, but still can have an effect on culture, economics, drug-resistance, animal welfare, and conservation efforts. Cleaveland et al.,
found that >61% of human pathogens, >77% of livestock pathogens and >90% of carnivore pathogens were multi-host pathogens [7]. Though multi-host pathogens are more common in the field of parasitology, single-host pathogens dominate the scientific literature (Figure 1.1). These estimates indicate the challenges and numerous opportunities to expand knowledge and collaboration between disciplines to better understand complexity in the dynamics of multi-host pathogens.
Figure 1.1 Comparison of trends in publications by year: (parasite or parasitology) and single-host versus (parasite or parasitology) and multi-host.

All figures presented in this chapter were created using indexed publications in Scopus© database, accessed March 11, 2016. These searches might not be a representation of the true efforts to address multi-host pathogens. The search terms were: (parasite or parasitology) and complexities, (parasite or parasitology) and multidisciplinary, (parasite or parasitology) and One-Health, (parasite or parasitology) and single-host, and (parasite or parasitology) and multi-host. Additional search terms other than the ones used here might identify more literature on multi-host pathogens. Also, using (parasite or parasitology) constrains the search to that type of pathogens, and particularly, we focused on those because *Neospora caninum* is identified as a parasite.
Interestingly, awareness of infectious disease systems’ complexity is illustrated in the increasing scientific literature (Figure 1.2) on the subject, yet the trend in publications by year using multidisciplinary approaches is slightly growing, including literature using the ‘One Health’ approach (Figure 1.3). One Health is defined as the collaborative effort of diverse disciplines operating at various spatial scales, from local to global, to reach optimal health for people, animals and the environment [8,9,10]. Although this concept has been promoted recently, the multidisciplinary thinking to better understand health-related complexity has a long historical trajectory. For example, veterinarians and anthropologists have joined forces to address complexities of the ‘real’ world of ethno-veterinary systems to design and implement socioculturally acceptable and ecologically and economically sound interventions to improve animal health and productivity [11]. Moreover, a new scientific field, conservation medicine, resulted from the integration of health and ecology disciplines and recognizes the health component of conserving biodiversity [12]. Accordingly, within the One Health framework, we sought collaboration with and engagement of farmers, veterinarians, ecologists, modelers, anthropologists, and conservationists to address the complexities involved in the study of the ecology of a multi-host pathogen, *N. caninum*, at the wildlife-livestock interface.
Figure 1.2 Historical trend in number of publications with words (parasite or parasitology) and complexity. Indexed publications in Scopus© database.
Figure 1.3 Comparison of trends in publications by year: (parasite or parasitology) and multidisciplinary versus (parasite or parasitology) and one-health. Indexed publications in Scopus© database.
Research on pathogen transmission at the wildlife-livestock interface not only promotes better understanding of complex disease dynamics but also integrative and adaptive management strategies for a One Health approach. In North America, the top three reported wildlife-livestock interfaces for infectious diseases are birds-poultry, artiodactyls-cattle and carnivores-cattle [13]. One area of concern for those interfaces is the difficulty of controlling diseases in domestic populations when wildlife species act as reservoirs for those pathogens [14]. Similarly, threats are posed to the conservation of wildlife populations from pathogens that circulate in domestic populations [15]. Consequently, feedback loops of interspecies pathogen transmission that allow pathogen persistence in a community quickly become a task of multidisciplinary scientific teams. An analysis made on indexed publications in Scopus© database by subject areas showed medicine (28%) at the forefront of literature on (parasite or parasitology) and complexity, multidisciplinary, and One-health (Figure 1.4). Conversely, agriculture and biological sciences is the area that showed the most literature on single-host (37%) and multi-host (31%) parasites, whereas veterinary medicine showed much less literature on single-host (2.3%) and multi-host (3.2%) parasites (Figure 1.4). While this search may be biased by the terms used, a skewed distribution can be seen. Figure 1.4 highlights subject areas that could potentially be integrated to better understand complexities of multi-host pathogens, such as computer science, and arts and humanities. For example, human dimensions research should be included in the study of the ecology of infectious diseases because human populations and their anthropogenic activities are part of the ecosystem in which disease dynamics occur and have an effect. Recognition of local knowledge and how that
local knowledge affects in the dynamics of infectious diseases may give us alternatives on how to incorporate infectious disease management for human development, animal welfare and the preservation of cultures [16,17]. Neglecting human dimension studies can mask the recognition and usefulness of the existing practical knowledge applied to infectious disease dynamics; thus, external interventions ignoring existing knowledge may cause conflicts [17]. In the case of N. caninum, our studied protozoan parasite capable of infecting a wide range of canid and ungulate species [18,19,20], the two main subject areas where literature is indexed in the Scopus© database are immunology and microbiology (32%), and veterinary (31%), and none in social science (Figure 1.5).
Figure 1.4 Number of publications by subject area. Indexed publications in Scopus© database. A query with words found in title, abstract and keywords: (parasite or parasitology) and complexity, (parasite or parasitology) and multidisciplinary, and (parasite or parasitology) and one-health, (parasite or parasitology) and single-host, (parasite or parasitology) and multi-host.
*N. caninum* was first isolated in 1984 from the brains of dogs with meningoencephalitis in Norway [21]. In 1988, it was recognized as a new genus, *Neospora* [22]. The importance of Neosporosis relates to the substantial economic losses to the cattle industry, both nationally and internationally, mainly derived from premature culling, reduced milk yields and abortions caused by the parasite [23,24]. *N. caninum* costs $843 million dollars annually in US dairy farms alone [23]. The well-documented domestic cycle involves domestic dogs and cattle. In addition to the domestic cycle, a wildlife cycle of the parasite has been identified in various part of the world (i.e. Europe, Oceania, America), making a wildlife-livestock interface plausible in the disease ecology of this protozoan parasite [19,25,26]. Prevention of *N. caninum* infection is based on reducing direct and indirect interactions between the definitive host (canids) and the intermediate host (ruminants), as well as maintaining a closed herd [27]. Also, because transplacental transmission (cow-calf) seems to be the primary source of maintaining the parasite within the herd as opposed to horizontal transmission (e.g. via colostrum and/or environment), vaccines have been suggested as a control strategy to limit infection [28]. However, at the moment, there is no vaccine on the market [29].
Figure 1.5 Number of publications of *Neospora caninum* by subject area. Indexed publications in Scopus© database. A query with words found in title, abstract and keywords: *Neospora* and *caninum*. 
Our goal was to better understand the ecology of *N. caninum* at wildlife-livestock interface in natural settings. As in many natural settings, our study area harbors diverse domestic and non-domestic species. This community structure is rarely investigated as an epidemiological continuum where each species might affect pathogen transmission or lack thereof. In this context, first, we estimated the true prevalence of three ruminant species by using Bayesian inference. We identified and discussed differences between apparent and true prevalence (TP). Differences in TP for some species suggest differences in the epidemiology of *N. caninum* for these co-located populations. Accurate measurement of prevalence and incidence in each host population could support ecosystem-based prevention and control of multi-host pathogens, impacting disease dynamics at the community level. Second, we evaluated the environmental phase of *N. caninum* shed in wild canid scats. Specifically, we focused on coyotes, as a recent dramatic increase of their ranges and population has enabled them to expand to the entirety of the US and Mexico, a vast area of Canada, and Central America [30]. Results suggested that the role of this environmental phase in the transmission to ruminants is likely minor. Finally, we evaluated the role of host species heterogeneity in the epidemiology of *N. caninum* circulating in a community. We identified differences in the role of immunity, age structure, and maternal and/or fetal antibody duration in three intermediate (ruminant) host species. Also, we estimated the species-specific contribution to the persistence of this pathogen in a community. Those estimates allow us to propose targeted control, but further evaluation is needed to confirm our findings.
1.1 References

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Chapter 2: Estimating Neospora caninum prevalence in wildlife populations using Bayesian inference

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A slightly different version has been published in Ecology and Evolution, in press
2.1 Abstract

Prevalence of disease in wildlife populations, which is necessary for developing disease models and conducting epidemiologic analyses, is often understudied. Laboratory tests used to screen for diseases in wildlife populations often are validated only for domestic animals. Consequently, the use of these tests for wildlife populations may lead to inaccurate estimates of disease prevalence. We demonstrate the use of Bayesian latent class analysis (LCA) in determining the specificity and sensitivity of a cELISA (VMRD®, Inc., Pullman, WA, USA) serologic test used to identify exposure to *Neospora caninum* (hereafter *N. caninum*) in three wildlife populations in southeastern Ohio, USA. True prevalence of *N. caninum* exposure in these populations was estimated to range from 0.1 to 3.1% in American bison (*Bison bison*), 51.0 to 53.8% in Père David’s deer (*Elaphurus davidianus*) and 40.0 to 45.9% in white-tailed deer (*Odocoileus virginianus*). The accuracy of the cELISA in American bison and Père David’s deer was estimated to be close to the 96% sensitivity and 99% specificity reported by the manufacturer. Sensitivity in white-tailed deer, however, ranged from 78.9 to 99.9%. Apparent prevalence of *N. caninum* from the test results is not equal to the true prevalence in white-tailed deer and Père David’s deer populations. Even when these species inhabit the same community, the true prevalence in the two deer populations differed from the true prevalence in the American bison population. Variances in prevalence for some species suggest differences in the epidemiology of *Neospora caninum* for these co-located populations. Bayesian LCA methods could be used as in this example to overcome some of the constraints on validating tests in wildlife species.
The ability to accurately evaluate disease status and prevalence in a population improves our understanding of the epidemiology of multi-host pathogen systems at the community level.

**Key-words:** antibody test, gold standard, prior distribution, probability intervals, test accuracy, true prevalence

### 2.2 Introduction

Surveillance for wildlife diseases is critical to our understanding of the emergence, transmission, persistence and control of infectious diseases at the interface of humans, domestic animals, and wildlife populations [1]. For example, describing how the community composition could contribute to the persistence and spread of a multi-host pathogen in an ecological system relies directly on the ability to accurately identify the pathogen in the various host populations. Also, accurate measurement of prevalence and incidence in each host population could support ecosystem-based prevention and control of multi-host pathogens, impacting disease dynamics at the community level. However, laboratory tests used to screen diseases in wildlife populations are generally developed for domestic animals and not validated for wildlife species [2]. Thus, the application of these tests for wildlife populations may result in inaccurate estimates of epidemiologic parameters. Subsequently, any investigation, analysis or description of a host-pathogen system (e.g., risk factor analysis, transmission modeling) in which estimated parameters are applied might reach biased conclusions [3,4].
Conventionally, the estimation of prevalence of disease in a population is based on validated tests [5]. The true prevalence (TP) of disease in a population is the proportion of truly exposed or infected animals, whereas apparent prevalence (AP) is the proportion of test-positive animals [6]. Therefore, the difference between AP and TP of a disease is a function of sensitivity (Se) and specificity (Sp) of the test, where Se is the probability of correctly classifying exposed or infected individuals and Sp is the probability of correctly classifying non-exposed or non-infected individuals. For example, if a test has 50% Sp, half of all non-exposed/ non-infected individuals will test positive (false positives) and will inflate the AP. Conversely, a test with 50% Se would incorrectly classify half of all exposed/ infected individuals (false negatives) and deflate the AP compared with the TP. The relationship among AP, TP, Se and Sp is TP=[AP+Sp-1]/[Sp+Se-1]. Thus, it is important to know the Se and Sp for a test to make correct conclusions about prevalence in a population.

These parameters are often estimated from an experimental population or a reference test [3]. For wildlife populations, an accurate reference test (referred to as the gold standard) is often unavailable, impractically invasive or prohibitively expensive. Moreover, validation of tests for wildlife populations using traditional field and laboratory methods is limited due to small numbers of individuals and populations, ethical restrictions, economical burden, and specialized management and housing requirements of wildlife species [2]. However, Gardner et al. (1996) describe the importance of validating a test for use in wildlife species even when that test has been validated for livestock. Fortunately, statistical methods can be applied to address the issue
of uncertain test accuracy in wildlife populations and to estimate prevalence in the absence of a gold standard.

Enoe, Georgiadis & Johnson [7] discuss the differences and limitations of commonly used statistical methods for maximum likelihood (ML) estimation and Bayesian inference. For instance, they argue that ML estimations rely on the assumption of large sample size; thus, confidence intervals are valid only if that assumption is met. In contrast, Bayesian inference is not restricted by large sample size assumptions (e.g., Normality), but an extra step specifying prior knowledge is required. In the case of wildlife health, large sample sizes are uncommon, but often many small studies or expert opinions can be found, making this an ideal area for use of Bayesian inference.

Bayesian latent class analysis (LCA) combines preliminary estimation of the expected disease prevalence and knowledge of the test performance characteristics with the likelihood of the observed data in order to infer the TP and test Se and Sp of the targeted population. Because a gold standard is not available, the true classification of an individual as infected or not infected is missing, thus analyses using that latent (or missing) data are called ‘latent class analysis’ [8,9,10]. As others have shown [11,12,13,14], this method uses prior knowledge regarding parameter estimates (a prior distribution) to circumvent the problem of having two observations (numbers of test positive and test negative animals) and three unknown parameters to estimate (TP, Se and Sp).

*Neospora caninum* is an excellent case study in which to apply these methods. *N. caninum* is one of the major causes of reproductive problems and abortions in dairy and
beef cattle worldwide [15]. This protozoan parasite was first recognized in 1984 in dogs in Norway [16]. Later, in 1988, it was proposed as a new genus, *Neospora* [17]. Currently, the described life cycle of *N. caninum* comprises sylvatic and domestic cycles [18,19]. In the United States, coyotes and dogs are believed to be the main definitive hosts and white-tailed deer and cows the main intermediate hosts [18,20]. Numerous investigations have advanced knowledge on host immunology, livestock-related economics, risk factors, and species exposure to *N. caninum* [21,22,23]. Although knowledge of exposure of a wide range of species has been described, little is known about the true population prevalence in different species, especially in wildlife; even less is known about *N. caninum*’s effects on the population dynamics of these wildlife species and its spread and persistence at the community level. Therefore, estimates of the TP in wildlife populations will advance this area.

Various diagnostic assays have been developed to evaluate *N. caninum* exposure and infection in multiple hosts [24]. However, most of these tests are applied in research environments; operational characteristics of the tests might limit their application in surveillance programs [25]. For example, time taken to perform tests such as histopathology and immunohistochemistry to assess *N. caninum* in tissue samples (i.e. aborted fetus, placenta and brain) [26], difficulty of obtaining necessary samples like aborted fetuses from wild ruminants, and technical involvement to identify antibodies in blood by the indirect fluorescent-antibody assay in various species [27] limit their use in wildlife surveillance programs [28]. The commercially available competitive enzyme-linked immunosorbent assay (cELISA) allows multi-species testing, timely results, easy
access, technical simplicity and an economical way to identify *N. caninum* exposure [29].
The cELISA serological test has a cut-off value, which maximizes the test Sp and Se for
domestic cattle populations [30]. However, uncertainty of cELISA accuracy in wildlife
populations should be taken into account when estimating prevalence.

Our goal was to infer the TP of *N. caninum* in managed Père David’s deer (*Elaphurus
davidianus*) and American bison (*Bison bison*) herds and a free-ranging white-tailed deer
(*Odocoileus virginianus*) population, which inhabit the same community in southeastern
Ohio, USA, by applying Bayesian LCA. We have hypothesized that TP will differ by
species and from the AP. Also, we used the TP to evaluate the Se and Sp of the
commercial cELISA kit (VMRD®, Inc., Pullman, WA, USA), which has been used
successfully in domestic populations and could identify parasite exposure in these
wildlife populations.

### 2.3 Materials and methods

#### 2.3.1 Study area and Population

The study area in the Ohio Appalachian bioregion intersects four counties
(Muskingum, Morgan, Noble, and Guernsey) and contains the largest conservation center
in North America, the International Center for the Preservation of Wild animals (DBA,
*the Wilds*). *The Wilds* specializes in captive breeding of rare and endangered species
including many endangered ruminant species, and encompasses approximately 9,250
acres (40.46 km²) of reclaimed mine land. The main vegetation is open grassland and
forest, (see [31] for detailed land structure and vegetation). The area surrounding *the*
Wilds is rural, and animal agriculture is the primary or is a supplemental source of income for many families in the area. Free-ranging wildlife species such as white-tailed deer (*Odocoileus virginianus*) and coyote (*Canis latrans*) are pests, tourist attractions, and a source of food and recreation for the local community. Within the Wilds’ property, fences limit interactions between livestock and captive wildlife (e.g., American bison and Père David’s deer), yet pathogen transmission is plausible between them. White-tailed deer and coyotes comingle with both captive populations, as both free-ranging species are capable of crossing many fence lines. This comingling unlocks pathways of pathogen transmission and persistence among Père David’s deer, American bison and white-tailed deer, our focal species. We have selected this area because it offers a natural laboratory with a complex wildlife-livestock interface that allows us to examine the disease dynamics of multi-host pathogen systems, such as *N. caninum*, at the community level.

2.3.2 Sample collection and testing

We designed a cross-sectional epidemiological study in which three wildlife species were sampled. We collected tail or jugular vein blood samples (10 ml per individual) during March and April of 2013 for 38 (23 females, 13 males, 2 unrecorded) Père David’s deer and 81 (52 females, 26 males, 3 unrecorded) American bison managed at the Wilds. The individuals were physically restrained and, once restrained, the procedure lasted about 10 minutes per animal. Individuals were sampled early in the morning to avoid heat stress and acutely stressed individuals were removed from the study. Thirty samples (27 females and 3 males) from free-ranging white-tailed deer were
obtained from the study area during Ohio’s hunting season in 2012 and 2013. Deer hunting season in Ohio extends from October to February. We collected 10ml of blood per deer directly from the heart, soon after death to avoid degradation of antibodies. Animal use protocols were reviewed and approved by the Ohio State University Institutional Animal Care and Use Committee. A white-tailed deer scientific collection permit was granted by the Division of Wildlife, Ohio Department of Natural Resources. Serum was extracted by centrifugation and stored at -20°C. All species’ serum was tested for *N. caninum* antibodies using a commercial cELISA kit (VMRD®, Inc., Pullman, WA, USA) at the Ohio Department of Agriculture Animal Disease Diagnostic Laboratory, Reynoldsburg, OH. The cELISA was performed according to manufacturer recommendations. Individual results were reported as percentage inhibition values; a value $\geq 30\%$ inhibition was considered a positive result and $<30\%$ a negative result, as that cut-off is currently used by the manufacturer and has been validated for cattle [30].

2.3.3 Statistical Analysis

Our analysis involved one test across three species to estimate three population parameters (Se, Sp and prevalence) for each species; thus, there were more parameters to estimate than degrees of freedom in the data [32]. Therefore, selection of accurate prior information was essential to minimize the effects of this constraint.

To set prior beliefs, informative and non-informative distributions were used. Informative distributions were based on peer reviewed literature estimates of prevalence, Se and Sp (See Table 2.1). When 95% confidence intervals were not provided directly by
the publication, intervals were approximated accordingly to the ‘score’ method, corrected for continuity [33,34] by using VassarStats: Website for Statistical Computation [35]. The parameter space in which prevalence, Se and Sp fluctuate is from 0 to 1, thus we used the beta distribution, which is an appropriate family to model uncertainty about parameters within this space [32]. For this analysis, transformation of the published estimates to beta parameters (α, β) was obtained by using the “betaExpert” function, available in the package ‘prevalence’ version 0.3.0 built for the statistical program R version 3.1.3 [36,37]. For non-informative distributions, a beta (α=1, β=1) distribution was used, which gives equal belief to each value within the parameter space. Here, the likelihood of the observed positive and negative test results and the latent data was calculated given prior distributions of prevalence, Se and Sp as specified in [32].
Table 2.1 Published estimates of informative priors.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>American bison ((Bison bison))</th>
<th>Père David’s deer ((Elaphurus davidianus))</th>
<th>White-tailed deer ((Odocoileus virginianus))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence (95% CI)</td>
<td>0.45 (0.02, 2.9) (^3)</td>
<td>25 (11.4, 45.2) (^6)</td>
<td>*47.8 (20, 88.2) (^2)</td>
</tr>
<tr>
<td>Sensitivity (95% CI)</td>
<td>96.4 (92.1, 98.5) (^1)</td>
<td>78.6 (47.7, 96.7) (^5)</td>
<td></td>
</tr>
<tr>
<td>Specificity (95% CI)</td>
<td>96.8 (92.4, 98.8) (^1)</td>
<td>99 (96.4, 99.7) (^5)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) (Baszler et al. 2001); \(^2\) (Dubey et al. 2009); \(^3\) (Dubey & Thulliez 2005); \(^5\) (Pruvot, Hutchins & Orsel 2014); \(^6\) (Sedlák & Bártová 2006); \(^7\) (Wapenaar et al. 2007)

*The mean prevalence was estimated from all 6 white-tailed deer published literature and the range of prevalence is used versus the 95% confidence interval.
To explore the sensitivity of our conclusions to our choice of prior distributions, eight different estimates (a1-a8) were specified for the American bison herd, five different estimates (b1-b5) were specified for the Père David’s deer herd and five different estimates (c1-c5) were specified for the white-tailed deer population. Each estimate combines different sets of prior distributions, progressing from non-informative to more informative (See Table 2.2).
Table 2.2 Model specifications.

<table>
<thead>
<tr>
<th>Model</th>
<th>Species</th>
<th>Prevalence</th>
<th>Sensitivity</th>
<th>Specificity cELISA</th>
<th>Specificity cELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>a1</td>
<td>American Bison</td>
<td>Beta(0.29, 63.20)</td>
<td>Beta(1,1)</td>
<td>Beta(1,1)</td>
<td></td>
</tr>
<tr>
<td>a2</td>
<td>American Bison</td>
<td>Beta(2.60, 16.92)</td>
<td>Beta(1,1)</td>
<td>Beta(1,1)</td>
<td></td>
</tr>
<tr>
<td>a3</td>
<td>American Bison</td>
<td>Beta(1,1)</td>
<td>Beta(104.45, 3.90)</td>
<td>Beta(95.27, 3.15)</td>
<td></td>
</tr>
<tr>
<td>a4</td>
<td>American Bison</td>
<td>Beta(1,1)</td>
<td>Beta(38.70, 4.78)</td>
<td>Beta(97.21, 0.98)</td>
<td></td>
</tr>
<tr>
<td>a5</td>
<td>American Bison</td>
<td>Beta(0.29, 63.20)</td>
<td>Beta(104.45, 3.90)</td>
<td>Beta(95.27, 3.15)</td>
<td></td>
</tr>
<tr>
<td>a6</td>
<td>American Bison</td>
<td>Beta(0.29, 63.20)</td>
<td>Beta(38.70, 4.78)</td>
<td>Beta(97.21, 0.98)</td>
<td></td>
</tr>
<tr>
<td>a7</td>
<td>American Bison</td>
<td>Beta(2.60, 16.92)</td>
<td>Beta(104.45, 3.90)</td>
<td>Beta(95.27, 3.15)</td>
<td></td>
</tr>
<tr>
<td>a8</td>
<td>American Bison</td>
<td>Beta(2.60, 16.92)</td>
<td>Beta(38.70, 4.78)</td>
<td>Beta(97.21, 0.98)</td>
<td></td>
</tr>
<tr>
<td>b1</td>
<td>Père David’s deer</td>
<td>Beta(5.65, 16.94)</td>
<td>Beta(1,1)</td>
<td>Beta(1,1)</td>
<td></td>
</tr>
<tr>
<td>b2</td>
<td>Père David’s deer</td>
<td>Beta(1,1)</td>
<td>Beta(6.85, 1.86)</td>
<td>Beta(103.83, 1.05)</td>
<td></td>
</tr>
<tr>
<td>b3</td>
<td>Père David’s deer</td>
<td>Beta(1,1)</td>
<td>Beta(2.51, 0.63)</td>
<td>Beta(190.31, 6.70)</td>
<td></td>
</tr>
<tr>
<td>b4</td>
<td>Père David’s deer</td>
<td>Beta(5.65, 16.94)</td>
<td>Beta(6.85, 1.86)</td>
<td>Beta(103.83, 1.05)</td>
<td></td>
</tr>
<tr>
<td>b5</td>
<td>Père David’s deer</td>
<td>Beta(5.65, 16.94)</td>
<td>Beta(2.51, 0.63)</td>
<td>Beta(190.31, 6.70)</td>
<td></td>
</tr>
<tr>
<td>c1</td>
<td>White-tailed deer</td>
<td>Beta(3.08, 3.36)</td>
<td>Beta(1,1)</td>
<td>Beta(1,1)</td>
<td></td>
</tr>
<tr>
<td>c2</td>
<td>White-tailed deer</td>
<td>Beta(1,1)</td>
<td>Beta(6.85, 1.86)</td>
<td>Beta(103.83, 1.05)</td>
<td></td>
</tr>
<tr>
<td>c3</td>
<td>White-tailed deer</td>
<td>Beta(1,1)</td>
<td>Beta(2.51, 0.63)</td>
<td>Beta(190.31, 6.70)</td>
<td></td>
</tr>
<tr>
<td>c4</td>
<td>White-tailed deer</td>
<td>Beta(3.08, 3.36)</td>
<td>Beta(6.85, 1.86)</td>
<td>Beta(103.83, 1.05)</td>
<td></td>
</tr>
<tr>
<td>c5</td>
<td>White-tailed deer</td>
<td>Beta(3.08, 3.36)</td>
<td>Beta(2.51, 0.63)</td>
<td>Beta(190.31, 6.70)</td>
<td></td>
</tr>
</tbody>
</table>
The distributions of TP, Se and Sp were calculated using BayesDiagnosticTest Version 3.9.1 Software Package [38]. Concisely, the latent data (numbers of true positive and false negative individuals) are first estimated, then these estimates are used to obtain the TP, Se and Sp of the targeted population by applying the Gibbs sampling algorithm. The equations and application of the Gibbs sampling algorithm used in this software are explicitly described in [32]. Inferences were based on 100,000 iterations after a discarded burn-in of 10,000 iterations. The assumptions of the Bayesian estimation procedure we used are convergence and independence. Convergence was assessed by calculating the Brooks Gelman Rubin (BGR) diagnostic; and independence was assessed with autocorrelation plots (all estimates were from procedures that converged and showed independence; diagnostics not shown in this manuscript) using WinBUGS 1.4 software [14,39].

To determine if the TP differs by species, mode and 95% probability interval posterior estimates of the three species pairwise TP comparison was calculated using the most precise prior information for each species.

2.4 Results

A total of 81 American bison, 38 Père David’s deer, and 30 white-tailed deer were sampled. The number of positive individuals was 1/81 for American bison, 26/38 for Père David’s deer and 11/30 for white-tailed deer when using the 30% inhibition as the cutoff value to discriminate between positive (≥ 30%) and negative (< 30%) samples.
The posterior distributions of prevalence, Se and Sp by species were summarized by displaying the mode and 95% probability intervals of each of the models (See Tables 2.3-2.5). The models were sensitive for prior distributions; thus, careful selection of prior distributions was needed for inference. To draw inferences on the prevalence, Se and Sp of our studied populations, models containing informative prior distributions were selected [American Bison (a5-a8); Père David’s deer (b4-b5); white-tailed deer (c4-c5)]. The TP ranges were 0.1 to 3.1%, 51.0 to 53.8% and 40.0 to 45.9% for the American bison, Père David’s deer and white-tailed deer, respectively. Se ranges were 90.7 to 97.3%, 95.9 to 99.9% and 78.9 to 99.9% for the American bison, Père David’s deer and white-tailed deer, respectively. Sp ranges were 98.2 to 99.9%, 96.7 to 99.9% and 97.3 to 99.9% for the American bison, Père David’s deer and white-tailed deer, respectively.
Table 2.3 American bison herd prior (mean and [95% confidence interval]) and posterior distributions (mode and [95% probability interval]).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a1</td>
<td>0.0045</td>
<td>non-informative</td>
<td>0.001</td>
<td>0.161</td>
<td>non-informative</td>
<td>0.988</td>
</tr>
<tr>
<td></td>
<td>[0.0002,0.029]</td>
<td></td>
<td>[2.79E-08, 0.024]</td>
<td>[0.024, 0.974]</td>
<td></td>
<td>[0.935, 0.998]</td>
</tr>
<tr>
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<td>0.133</td>
<td>non-informative</td>
<td>0.108</td>
<td>0.102</td>
<td>non-informative</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>[0.043, 0.316]</td>
<td></td>
<td>[0.015, 0.264]</td>
<td>[0.006, 0.842]</td>
<td></td>
<td>[0.935, 0.999]</td>
</tr>
<tr>
<td>a3</td>
<td>non-informative</td>
<td>0.011</td>
<td>0.964</td>
<td>0.973</td>
<td>0.968</td>
<td>0.982</td>
</tr>
<tr>
<td></td>
<td>[5.37E-04, 0.059]</td>
<td></td>
<td>[0.921, 0.985]</td>
<td>[0.921, 0.990]</td>
<td></td>
<td>[0.953, 0.995]</td>
</tr>
<tr>
<td>a4</td>
<td>non-informative</td>
<td>0.011</td>
<td>0.890</td>
<td>0.897</td>
<td>0.990</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>[0.001, 0.070]</td>
<td></td>
<td>[0.79, 0.98]</td>
<td>[0.778, 0.963]</td>
<td></td>
<td>[0.975, 0.999]</td>
</tr>
<tr>
<td>a5</td>
<td>0.0045</td>
<td>0.001</td>
<td>0.964</td>
<td>0.972</td>
<td>0.968</td>
<td>0.982</td>
</tr>
<tr>
<td></td>
<td>[0.0002,0.029]</td>
<td></td>
<td>[2.09E-08, 0.017]</td>
<td>[0.921, 0.985]</td>
<td></td>
<td>[0.951, 0.994]</td>
</tr>
<tr>
<td>a6</td>
<td>0.0045</td>
<td>0.010, 0.011</td>
<td>0.890</td>
<td>0.907</td>
<td>0.990</td>
<td>0.996</td>
</tr>
<tr>
<td></td>
<td>[0.0002,0.029]</td>
<td>[4.07E-08, 0.022]</td>
<td>[0.79, 0.98]</td>
<td>[0.783, 0.964]</td>
<td></td>
<td>[0.971, 0.999]</td>
</tr>
<tr>
<td>a7</td>
<td>0.133</td>
<td>0.016</td>
<td>0.964</td>
<td>0.973</td>
<td>0.968</td>
<td>0.987</td>
</tr>
<tr>
<td></td>
<td>[0.043, 0.316]</td>
<td></td>
<td>[0.921, 0.985]</td>
<td>[0.920, 0.990]</td>
<td></td>
<td>[0.954, 0.995]</td>
</tr>
<tr>
<td>a8</td>
<td>0.133</td>
<td>0.031</td>
<td>0.890</td>
<td>0.910</td>
<td>0.990</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>[0.043, 0.316]</td>
<td></td>
<td>[0.79, 0.98]</td>
<td>[0.773, 0.962]</td>
<td></td>
<td>[0.977, 0.999]</td>
</tr>
</tbody>
</table>
Table 2.4 Père David’s deer herd prior (mean and [95% confidence interval]) and posterior distributions (mode and [95% probability interval]).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>b1</td>
<td>0.25</td>
<td>0.235</td>
<td>non-informative</td>
<td>0.619, 0.679, 0.877</td>
<td>non-informative</td>
<td>0.264</td>
</tr>
<tr>
<td></td>
<td>[0.114, 0.452]</td>
<td>[0.103, 0.444]</td>
<td></td>
<td>[0.032, 0.976]</td>
<td></td>
<td>[0.035, 0.563]</td>
</tr>
<tr>
<td>b2</td>
<td>non-informative</td>
<td>0.779</td>
<td>0.786</td>
<td>0.882</td>
<td>0.990</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>[0.594, 0.986]</td>
<td>[0.477, 0.967]</td>
<td></td>
<td>[0.638, 0.974]</td>
<td></td>
<td>[0.964, 0.999]</td>
</tr>
<tr>
<td>b3</td>
<td>non-informative</td>
<td>0.715, 0.742</td>
<td>0.800</td>
<td>0.999</td>
<td>0.966</td>
<td>0.968</td>
</tr>
<tr>
<td></td>
<td>[0.549, 0.979]</td>
<td>[0.298, 0.989]</td>
<td></td>
<td>[0.642, 0.999]</td>
<td></td>
<td>[0.935, 0.983]</td>
</tr>
<tr>
<td>b4</td>
<td>0.25</td>
<td>0.538</td>
<td>0.786</td>
<td>0.959</td>
<td>0.990</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>[0.114, 0.452]</td>
<td>[0.403, 0.666]</td>
<td></td>
<td>[0.787, 0.990]</td>
<td></td>
<td>[0.964, 0.999]</td>
</tr>
<tr>
<td>b5</td>
<td>0.25</td>
<td>0.511</td>
<td>0.800</td>
<td>0.999</td>
<td>0.966</td>
<td>0.967, 0.968</td>
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<tr>
<td></td>
<td>[0.114, 0.452]</td>
<td>[0.383, 0.647]</td>
<td></td>
<td>[0.839, 0.999]</td>
<td></td>
<td>[0.935, 0.983]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[0.932, 0.986]</td>
</tr>
</tbody>
</table>
Table 2.5 White-tailed deer herd prior (mean and [range]) and posterior distributions (mode and [95% probability interval]).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>c1</td>
<td>0.478</td>
<td>0.378, 0.516</td>
<td>non-informative</td>
<td>0.254</td>
<td>non-informative</td>
<td>0.664, 0.744</td>
</tr>
<tr>
<td></td>
<td>[0.20, 0.882]</td>
<td>[0.152, 0.817]</td>
<td></td>
<td>[0.025, 0.929]</td>
<td></td>
<td>[0.086, 0.973]</td>
</tr>
<tr>
<td>c2</td>
<td>non-informative</td>
<td>0.397</td>
<td>0.786</td>
<td>0.902</td>
<td>0.990</td>
<td>0.999</td>
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<tr>
<td></td>
<td>[0.257, 0.859]</td>
<td>[0.477, 0.967]</td>
<td></td>
<td>[0.4668, 0.9691]</td>
<td></td>
<td>[0.964, 0.997]</td>
</tr>
<tr>
<td>c3</td>
<td>non-informative</td>
<td>0.423</td>
<td>0.800</td>
<td>0.999</td>
<td>0.966</td>
<td>0.969</td>
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<tr>
<td></td>
<td>[0.222, 0.916]</td>
<td>[0.298, 0.989]</td>
<td></td>
<td>[0.379, 0.9985]</td>
<td></td>
<td>[0.935, 0.983]</td>
</tr>
<tr>
<td>c4</td>
<td>0.478</td>
<td>0.459</td>
<td>0.786</td>
<td>0.789, 0.859</td>
<td>0.990</td>
<td>0.999</td>
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<tr>
<td></td>
<td>[0.20, 0.882]</td>
<td>[0.273, 0.730]</td>
<td></td>
<td>[0.477, 0.967]</td>
<td></td>
<td>[0.964, 0.997]</td>
</tr>
<tr>
<td>c5</td>
<td>0.478</td>
<td>0.401</td>
<td>0.800</td>
<td>0.999</td>
<td>0.966</td>
<td>0.973</td>
</tr>
<tr>
<td></td>
<td>[0.20, 0.882]</td>
<td>[0.243, 0.747]</td>
<td></td>
<td>[0.442, 0.999]</td>
<td></td>
<td>[0.935, 0.983]</td>
</tr>
</tbody>
</table>
Models a7, b4, c4 were selected to compare AP and TP and to test whether TP differed by species. Selection of these three models was grounded on informed prior distributions and two main factors 1) prior data was from species belonging to the same family, and/or 2) population management was similar in the herd used in the prior estimate. AP was greater than TP in Père David’s deer and less than TP in white-tailed deer, while only a slight difference was seen between AP and TP in American bison (See Table 2.6).

Table 2.6 Apparent prevalence versus true prevalence.

<table>
<thead>
<tr>
<th>Species</th>
<th>Apparent Prevalence</th>
<th>True Prevalence*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[95% CI]</td>
<td>[95% PI]</td>
</tr>
<tr>
<td>American Bison</td>
<td>1.23 % [0.06, 7.6]</td>
<td>1.6% [0.6, 7.8]</td>
</tr>
<tr>
<td>Père David’s deer</td>
<td>68.4 % [51, 82]</td>
<td>53.8% [40.3, 66.6]</td>
</tr>
<tr>
<td>White-tailed deer</td>
<td>36.7 % [20.5, 56]</td>
<td>45.9 % [27.3, 73]</td>
</tr>
</tbody>
</table>

*The mode of models a7, b4 and c4 were selected to represent true prevalence.
We found that TP differed between American bison and the two deer populations. However, the TP was similar between Père David’s deer and white-tailed deer (Figure 2.1, Table 2.7).

Figure 2.1 Compared true prevalence by species.

Model a7, b4 and c4 were selected to represent true prevalence.
Table 2.7 The three species pairwise true prevalence comparison.

<table>
<thead>
<tr>
<th>Difference of the pairwise species comparison</th>
<th>Mode [95%PI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Père David’s deer- American Bison</td>
<td>0.49 [0.36-0.63]</td>
</tr>
<tr>
<td>White-tailed deer- American Bison</td>
<td>0.46 [0.23-0.69]</td>
</tr>
<tr>
<td>White-tailed deer- Père David’s deer</td>
<td>-0.11 [-0.30-0.22]</td>
</tr>
</tbody>
</table>

Models a7, b4 and c4 were selected to determine the difference of the TP by species.

2.5 Discussion

In our study, we estimated TP, Se and Sp of *N. caninum* exposure in three wildlife species in southeastern Ohio, USA. The accuracy of the commercially available cELISA kit (VMRD®, Inc., Pullman, WA, USA) in these species, using the 30% cutoff value, was close to the 96% Se and 99% Sp that the manufacturer specifies. High Sp was observed for all species tested, which makes this test good for diagnostic purposes. However, low Se was observed for white tailed deer, thus surveillance programs need to take into account that some infected animals may not test positive using the 30% inhibition cut-off criterion, or more work should be done in this species to establish the true Se of the test compared with a gold-standard.

Next, we compared AP versus TP. Apparent prevalence overestimated TP in Père David’s deer and underestimated TP in white-tailed deer, while only a slight difference
was seen between AP and TP in American bison. Despite the availability of methods to measure disease prevalence when diagnostic uncertainties are unknown, AP continues to be reported [40,41]. Apparent prevalence measured over time might provide the trajectory of a disease in a population. However, AP might hinder control or eradication programs. For example, a study on bovine tuberculosis in harvested white-tailed deer in Michigan showed that AP used as a metric in the surveillance program to assess progress towards disease eradication underestimated TP. Hence false negative individuals were hindering a control program and a risk to hunters’ health [4]. Because the goal of surveillance programs is to detect infected individuals, a more sensitive test would be preferred, especially when prevalence is low, or the test results must be adjusted. Additionally, if AP is skewed differently for different species as appears to be the case here, conclusions of meta-population transmission models may be inaccurate if they use the skewed data across populations.

Finally, although these species inhabit the same community, we found that TP differed between American bison and the two deer populations, but TP was similar between Père David’s deer and white-tailed deer. Assuming that ruminant’s exposure to *N. caninum* from environmental contamination is homogenously distributed across the community, the variations seen among species might relate to difference in management (e.g., number of individuals transferred out of and into the population), differences in behavior (e.g., matriarchal structure of a population) and differences in immunity (e.g., host susceptibility or efficiency of vertical transmission). In 2003, a study estimated an overall cattle sero-positivity of 4.7% in Ohio and 9.2% in southeastern Ohio [42], thus
Neospora-endemic regions such as ours might further benefit from the correct classification of the health status of individuals. Correct classification might add better understanding on the role of types of host (e.g., maintenance host, spillover host), as well as quantifying the between and within species transmission on the persistence of a pathogen in a community [43].

Although Bayesian LCA overcomes many constraints of test validation in wildlife species, careful selection of prior distributions is needed because of unidentifiability (more parameters to estimate than degrees of freedom), which may lead to many distinct values of the parameters. For instance, we found that estimation using non-informative combinations of prior distributions (those giving equal belief to a prevalence of zero and a prevalence of one) produced very different estimates of prevalence, Se and Sp, than did our informed prior distributions (see Tables 2.3-2.5). Thus, we used estimates of the population parameters where prior distributions were informative, i.e. they restricted the parameter space to values that seem reasonable based on literature. When we used different informed prior distributions, parameter estimates did not vary widely.

In our study, previous information on prevalence was available for the three species studied. However, prior information for Se and Sp was obtained from experimental data studying cows, which are a reasonable surrogate for American bison as both belong to the Bovidae family, and a statistical validation studying elk, which are a reasonable surrogate for the two deer populations, as all belong to the Cervidae family.

In terms of the quantification of N. caninum exposure in wildlife populations, a variety of serological tests have been implemented, nevertheless this diversity disfavors
the comparison of the prevalence estimates, due to the various methods and cut-off values used among researchers [29,44]. Additionally, uncertainty around those estimates is often unidentified.

Modeling the uncertainty about the values for test accuracy and prevalence with probability provides a rational view of dealing with incomplete knowledge or knowledge that may peripherally influence beliefs about test characteristics [7]. For example, we accounted for uncertainty on cross-reaction by allowing a range of probabilities in prior distributions of Sp. Cross-reaction with antibodies produced by antigenically-related parasites such as *Toxoplasma gondii, Sarcocystis cruzi, Sarcocystis hominis*, and *Sarcocystis hirsute* could reduce test Sp, however the use of the 65-kDa *N. caninum* tachyzoite antigen and a monoclonal antibody (MAb 4A 4-2) in the competitive ELISA (VMRD®, Inc., Pullman, WA, USA) to detect antibodies in sera instead of whole tachyzoite antigens used in indirect ELISA and IFA tests has been suggested to alleviate this problem [45]. Nevertheless, a serological cross-reaction with *Hammondia heydorni* has not been evaluated [46].

To our knowledge, the cELISA (VMRD®, Inc., Pullman, WA, USA) for *N. caninum* has been experimentally validated for cattle, statistically validated for elk and partially validated for dogs through the work of Baszler *et al.*[30], Pruvot *et al.* [47], King *et al.* [48] and Capelli *et al.* [49]. Our results expand validation of the use of this test for white-tailed deer, American bison and Père David’s deer. The geographical distribution and abundance of white-tailed deer in America, in addition to hunting practices, make this potential intermediate host a valuable target species for understanding and controlling *N.*
caninum at the wildlife-livestock interface [46]. With regard to American bison and Père David’s deer, conservation efforts might be hindered by the persistence of this pathogen in their populations. Thus, better estimates of exposure might better explain the role of these species in the transmission cycle of N. caninum and the effects of the pathogen on reproduction, not only at the population level but also at the community level.

Variation of N. caninum prevalence among cohabiting species demonstrates likely differences in disease dynamics for the different species; thus providing evidence for the importance of community-based approaches to understanding transmission and persistence of multi-host pathogens.

2.6 Acknowledgments

We would like to thank the hunters, the volunteer field assistants and the Wilds for granting research permission and collaboration. Also, we would like to thank the Ohio Department of Agriculture Animal Disease Diagnostic Laboratory, Reynoldsburg, OH for performing the serological test. We are grateful for the great discussions provided by Laura Pomeroy and the Disease Ecology and Computer Modelling Laboratory group. The protocol number for the Ohio State University Institutional Animal Care and Use Committee was 2012A0000154 and the Wild Animal Permit-Scientific Collection, Division of Wildlife, Ohio Department of Natural Resources was 14-266. This research has been financially supported by grants from OSU CARES and Columbus Zoo & Aquarium. VMRD, Inc (Pullman, WA, USA) did not contribute to any stage of the study or document writing.
2.7 Data accessibility

Data is available and clearly written in the manuscript.

2.8 References


44. Dubey JP, Jenkins MC, Kwok OCH, Zink RL, Michalski ML, et al. (2009) Seroprevalence of Neospora caninum and Toxoplasma gondii antibodies in white-
tailed deer (Odocoileus virginianus) from Iowa and Minnesota using four serologic tests. Veterinary Parasitology 161: 330-334.


Chapter 3: Spatial distribution of *Neospora caninum* and morphologically similar oocysts shed by wild canid hosts into the environment

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3.1 Abstract

The role of wild canids in the dynamics of infectious diseases, specifically Neospora caninum, that spill over to ruminant species mainly has been studied in experimental settings but there are few studies of these dynamics in natural settings. We designed a non-invasive epidemiological study to collect wild canids’ scats across an ecological system. Visual and microscopic fecal examination and molecular analysis were used to detect the presence of N. caninum and other coccidian DNA shed in wild canid scats and confirm host species identification. Relative abundance of carnivores, cattle density, land use type, feces freshness and weather conditions were analyzed to determine if any association could be drawn between coccidian DNA detection and these explanatory variables. Our molecular results show a prevalence of 18% (95% CI: 13.8-22.7%) of coccidian DNA. Of those positive samples, 1.1% (95% CI: 0.35%-3.0%) tested positive for H. heydorni and none tested positive for N. caninum. This results in a 0% prevalence estimate for N. caninum with a maximum prevalence of 7.2% from these data. Morphologically identified wild canid scats belonged to 78.9% coyote and 19.6% red fox and the proportion of coccidian DNA positive scats did not differ between canid species. Temperature and fecal freshness showed a statistically significant relationship with the probability of detecting coccidian DNA. Land use type, relative canid abundance and density of cattle were not associated with the distribution of coccidian DNA positive samples. To our knowledge, this study is the first to survey the environmental phase of N. caninum shed in wild canid scats and suggests that the role of this environmental phase in the transmission to ruminants is likely minor. Further epidemiological studies in natural
settings might describe complex interactions between both weather conditions and diversity of hosts that drive the transmission of infectious diseases at the wildlife-livestock interface.

**Key-words:** Sarcocystidae, coccidian oocysts, coyote, fox, weather, canid abundance

### 3.2 Introduction

*Neospora caninum* infection is one of the major causes of reproductive problems and abortions in cattle worldwide [1]. This protozoan parasite was first isolated in 1984 from the brains of dogs with meningoencephalitis in Norway [2]. In 1988, it was recognized as a new genus, *Neospora* [3]. The importance of the disease relates to the economic losses to cattle production at both national and international levels [4]. Abortions linked to *N. caninum* are the main source of these economic losses, but the parasite can also cause economic loss through decreased milk production, increased culling and reduced value of breeding stock [5].

Experimental and epidemiological investigations have suggested two main routes of transmission between the intermediate and definitive hosts in the complex life cycle of *Neospora caninum* [1]. Maintenance of the parasite in the intermediate (ruminant) host herd seems to occur primarily by vertical transmission [6,7], while subsequent reintroduction of the parasite to the herd has been suggested to occur primarily horizontally, through environmental contamination of oocysts by the definitive (canine) host [8,9,10]. The well-documented domestic cycle involves domestic dogs and cattle. In
addition to the domestic cycle, a wildlife cycle of the parasite has been identified in
various part of the world (i.e. Europe, Oceania, America), making a wildlife-livestock
interface plausible in the disease ecology of this protozoan parasite [11,12,13].

Research on pathogen transmission at the wildlife-livestock interface not only
promotes better understanding of complex disease dynamics but also integrative and
adaptive management strategies for a One Health approach. One Health is defined as the
collaborative effort of diverse disciplines operating at various spatial scales, from local to
global, to reach optimal health for people, animals and the environment [14,15,16]. The
idea of the One Health approach, in this context, is to seek collaboration with and
engagement of farmers, veterinarians, ecologists, economists, modelers, anthropologists,
and conservationists to address the complexities involved in the interrelatedness of all
aspects of human, animal, and environmental health in a community. A recent literature
review using a scoping framework indicates that, in North America, the top three
reported wildlife-livestock interfaces for infectious diseases are birds-poultry,
artiodactyls-cattle and carnivores-cattle [17]. In North America, the presence of coyotes
and foxes has been considered a risk factor for cattle and feral swine neosporosis [18,19].
However, the role of free-ranging carnivores (e.g., coyote) in the epidemiology of
ungulate neosporosis and the dynamics of *N. caninum* oocyst excretion in definitive hosts
(e.g., dog, coyote, fox) have yet to be studied.

Historically, coyotes have been restricted mostly to the prairies of central North
America. A recent dramatic increase of coyote ranges and population has enabled them to
expand to the entirety of the US and Mexico, a vast area of Canada, and Central America
[20]. Therefore, an increase in the probability of contact between coyotes and livestock is expected [21,22]. An indication of this probability of contact is cattle losses by predation, which have increased from 1991 (2.4%) to 2010 (5.5%) in the US, of which approximately 90% of this predation was due to coyotes [23,24]. In Ohio, the coyote population has increased threefold since 1990 and cattle losses by predation have increased from 0.9% in 1995 to 5.6% in 2010 [24,25,26]. Though predation is an obvious result of the expanding coyote population, coyotes also influence livestock production through their roles as disease hosts and vectors. On the other hand, coyotes serve an essential ecological function of regulating populations of species, such as rodents and foxes, which can also serve as hosts and vectors of disease. Furthermore, coyotes serve as a bio-control for overabundant species, such as Canada geese and white-tailed deer [27]. Sacks and Neale [28], for example, described the role of wild prey biomass in decreasing sheep predation by coyotes, which highlights the importance of healthy food web dynamics in the overall sustainability of an ecosystem.

Previous and ongoing investigations in southeastern Ohio have reported ruminants that are seropositive for *Nesopora caninum*, such as Pere David’s deer (*Elaphurus davidianus*), Bison (*Bison bison*), domestic cattle (*Bos taurus*) and white-tailed deer (*Odocoileus virginianus*) [29,30,31,32]. Although a previous pilot study identified coccidia in a small sample (n = 29) of coyote feces by microscopic examination, this study did not confirm the parasite identification using molecular methods rather only *N. caninum* was ruled out [33]. The aim of the current study was to determine the role of wild carnivores’ scat in the dynamics of *Nesopora caninum* transmission that may spill
over to ruminant species in southeastern Ohio by: a) estimating wild canid relative abundance; b) assessing the prevalence of the environmental phase of *N. caninum* and other DNA of coccidian oocysts shed by wild canid hosts; and c) measuring association between the spatial distribution of DNA of coccidian oocysts and proposed risk factors for *N. caninum* (cattle density, canid relative abundance, land use).

### 3.3 Material and methods

#### 3.3.1 Study area and population

Our study area in the Ohio Appalachian bioregion intersects four counties (Muskingum, Morgan, Noble, and Guernsey) and includes the largest conservation center in North America, the International Center for the Preservation of Wild animals (DBA, *the Wilds*). The area surrounding *the Wilds* is rural; livestock farms include cattle (*Bos taurus*), sheep (*Ovis aries aries*), and goats (*Capra aegagrus hircus*). In addition, farms keep domestic dogs mainly as companions or livestock guards. Wild carnivores such as coyote and fox in the area are part of free ranging wildlife that are considered pests, tourist attractions, and a recreation source for the local community. We have selected this region because it allows us to explore the disease ecology of multi-host pathogens such as *Neospora caninum* and to begin to untangle the role of species such as the coyote at the wildlife-livestock interface. Within this region, the area selected for this study was based on the home range of coyotes [3 to 42 km², average 17.5 km²] [34] and consisted of five concentric buffers (5 km radius each buffer) around the property line of *the Wilds*. 
(adding to a total of 25 km radius) and centered around the Père David’s deer enclosure, see Figure 3.1.

Figure 3.1 Study area in southeast Ohio, USA.

Five concentric rings were drawn based on estimates of coyote’s home range to establish the study area. Buffers have a radius from 5 km to 25 km (Area ~1964 km2 (~758mi2)).
3.3.2 Study design and sample collection

We designed a spatial sampling strategy to determine wild canid relative abundance and the environmental prevalence and distribution of coccidian parasites such as *N. caninum* by using non-invasive fecal collection. Sampling took place from May to August 2014. At this time of the year in the US Midwest (Ohio), coyotes should have fixed home ranges due to rearing pups in dens [20]. Therefore, an assumption of independence for the analysis was made for samples collected between locations that were at least 5 km distant from each other. Coyotes are known to frequent linear features like roads, trails, fire breaks, and field edges, so these features were surveyed within our study area [35]. Transects (linear features) were selected at random from the available linear features on properties in the study area where owners agreed to participate in the study. Fifty-six transects corresponding to a total of 89.4 km were surveyed for wild canid scats, and included *the Wilds* and twenty-nine farm or recreational properties. Transect sizes ranged from 0.45 to 4.5 km. Each transect was surveyed twice by at least two researchers as follows: On the first day, transects were walked to clear any scat from the area. After 14 days, another sample collection was completed along transects.

Upon collection of feces, GPS coordinates were recorded and a scat’s freshness measurement was estimated as follows: a numerical scale was assigned based on color, wetness and presence of fecal material (i.e., metabolized prey tissue versus undigested material such as hair, bones, and feathers). Black, wet and presence of fecal material was considered a one (fresh); white, dry and absence of fecal material was considered three (old) and all others were classified as two (intermediate), see Figure 3.2. Additionally,
morphological characteristics described by Halfpenny [36] were used to identify the host species and a certainty level was assigned (70%, 90% and 100 %). To confirm our accuracy in identifying host species, molecular analysis on a subset of samples was successfully employed as described in Section 2.3.1.

Figure 3.2 Scat’s freshness measurement.

(Top) black, wet and presence of fecal material was considered a one (fresh); (Bottom) white, dry and absence of fecal material was considered three (old) and (Middle) all others were classified as two (intermediate).
The average temperature, humidity and precipitation for the 15 days prior to fecal collection were gathered from the National Oceanic and Atmospheric Association (http://www.ncdc.noaa.gov/cdo-web/) database accessed in November 4th 2014, station Zanesville municipal airport OH US, to determine the weather conditions that the feces were exposed to prior to collection. Land use data in the area of collection were recorded by the researchers based on visual inspection as: crop (cultivated agricultural use), pasture (livestock grazing or unoccupied grassland) or woods (forested). Finally, a survey of the property owner was implemented to obtain the number of cows per farm and number of acres per farm of the sampled transects.

3.3.3 Molecular analysis

3.3.3.1 Restriction enzyme analysis for host genetic identification

To confirm our morphological species identification of wild canid scats, we analyzed a section of mitochondrial DNA (mtDNA) of a subset of 50 coccidia-positive and 53 coccidia-negative scats. For collected scats, we extracted DNA from 1g of feces using QIAamp DNA stool Mini Kit (Qiagen, Hilden, Germany). A purification and concentration step was done by the method of ethanol precipitation [37]. For positive controls, DNA was extracted from whole blood (100 µl) banked samples of coyote (Canis latrans), red fox (Vulpes vulpes) and grey fox (Urocyon cinereoargenteus), provided by USDA Wildlife Services, National Wildlife Disease Program, using Qiagen DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany). We modified the method of digestion enzyme analysis developed by Adams et al. [38]. ScatID primers developed by
Adams et al. [38] were used to amplify 200 base pairs (bp) of the cytochrome b region of mtDNA in 20µl PCR reactions consisting of a final concentration mix of 200µM dNTP, 0.5µM forward primer, 0.5µM reverse primer, 1X Phusion HF Buffer, Phusion DNA polymerase 0.2µl (1.0 units/50µl PCR) (Phusion® High-Fidelity PCR Kit; New England BioLabs Inc., Ipswich, MA.), and template DNA 2.3µl (DNA concentration ranged 0.5-8ng/µl). PCR conditions were set on a Dyad PTC-220 Thermal Cycler (MJ Research, Inc., Waltham, MA) as follows: initial denaturation at 98°C for 30 sec; 35 cycles of 98°C for 10 sec, 50°C for 45 sec, 72°C for 15 sec; and final extension of 72°C for 5 min. A positive and a negative control (ultrapure DNase/RNase–free dH2O) were included in each batch. Amplified products were visualized on 2% standard agarose (IBI Scientific®, Kapp Court Peosta, IA) gels stained with ethidium bromide solution. Then, to differentiate between red fox and coyote or grey fox, 6.7µl of PCR product was digested with 1X CutSmart® buffer 0.8µl and 0.5 µl (1.0 units/50µl) TAQαI restriction enzyme (New England Biolabs Inc., Ipswich, MA.) in a 65°C dwell for 2 hours (8µl final volume) and inactivated at 80°C for 20 min. Digested products were visualized on 3% standard agarose (IBI Scientific®, Kapp Court Peosta, IA) gels stained with ethidium bromide solution. Finally, in order to differentiate between coyote and grey fox 6.8 µl of PCR product was digested with 1X CutSmart® buffer 0.8µl and 0.4 µl (1.0 units/50µl) HINF I restriction enzyme (New England Biolabs Inc., Ipswich, MA.) at 37°C overnight (8µl final volume) then inactivated at 80°C for 20 min. Digested products were visualized on 3% standard agarose (IBI Scientific®, Kapp Court Peosta, IA) gels stained with
ethidium bromide solution. Separated fragments were compared in size to a molecular 50 base pair DNA ladder (Invitrogen™).

3.3.3.2 Fecal examination and molecular identification of parasites

Feces were collected and stored at 4°C in individual sealed plastic bags [39] until processing (2-3 days). Sheather’s sugar solution/light microscopy test [40] was used to identify oocysts matching the morphologic and morphometric characteristics of *N. caninum* [39,40]. Other parasites and ova were recorded but not enumerated. For differentiation of *N. caninum* oocysts from other morphologically similar coccidia such as *Hammondia* spp., and *Toxoplasma gondii* [41], oocysts were isolated from 5g of feces by standard sucrose fecal flotation and placed in 6mL of 2.5% potassium dichromate, allowed to sporulate at room temperature overnight, and stored at 4°C until DNA extraction (7-13 months).

DNA extraction and parasite PCR methods are described in Sinnott et al. (Unpublished results). Briefly, detection of coccidian DNA by PCR with melting curve analysis (PCR-MCA) was performed using the universal coccidian primer cocktail developed by Lalonde and Gajadhar [42] to amplify a ~315bp region of 18S rDNA. Detection of coccidian DNA from our samples via this assay (hereafter, referred to as “coccidian DNA”) may have included detection coccidian oocysts associated with animal and human health, but also other coccidian species passing through the carnivore host due to the ingestion of the intermediate host. Coccidian DNA positive samples underwent further identification, using species-specific primers: Np6+/Np21+ targeting the Nc5
region of *N. caninum* [43], and JS4/JS5 targeting the ITS1 region of *H. heydorni* [44].

Results from these assays will, hereafter, be referred to as “*N. caninum* DNA” and “*H. heydorni* DNA”. A subset (n=3) of coccidian DNA positive amplicons, negative for both *N. caninum* and *H. heydorni* DNA, were further analyzed. These templates and applicable primers were submitted to The Ohio State University Plant-Microbe Genomics Facility for Big Dye Terminator Cycle Sequencing on a 3730 DNA Analyzer (Applied Biosystems). The resulting sequences were aligned using Vector NTI® software (Thermo Fisher Scientific). The DNA sequences were compared to published sequences using BLAST analysis available through the NCBI database and website.

3.3.4 Statistical and Spatial Analysis

To estimate relative abundance the scat deposition rate per km per day was used. The rate of fecal deposition per km per day (R) is calculated as \( R = \frac{S}{(L \times D)} \), where \( S \) is the number of feces found on the second walk in the transect, \( L \) is the length of transect and \( D \) is the number of days between the two observations [45]. Cattle density was estimated as the number of cows per farm acres.

We calculated the potential maximum prevalence of *N. caninum* DNA when all samples are negative by applying the equation described by Thrusfield [46], implemented in R version 3.1.3 [47]. In this equation, the population size \( (N=2223) \) was calculated as the total number of 1km\(^2\) hexagon shapes drawn using the Repeating Shapes file for ArcGis 9.3 [48] and the sample size was the number of hexagons occupied by the sampled transects \( (n=40) \). We approximated two 95% confidence intervals for the
coccidian DNA and *H. heydorni* DNA prevalences according to the ‘score’ method [49,50] as implemented in the ‘prevalence’ package in R [51].

To measure agreement beyond chance between *Neospora*-like oocysts microscopic visualization and coccidian DNA detection we calculated the Cohen’s Kappa coefficient [52], as implemented in ‘psych’ package in R [53]. For host identification, to determine how well observers’ certainty levels of the morphology classification matched results from the molecular analysis we performed the exact multinomial test [54], as implemented in ‘XNomial’ package in R [55]. To evaluate if a difference in proportion of coccidian DNA positive scats was statistically significant between morphologically identified wild canid scats species, we performed a Pearson’s Chi-squared test with Yates’ continuity correction.

To determine if detection of coccidian DNA could be affected by the weather conditions to which the wild canid scats were exposed prior to collection and the freshness of the scats at the time of collection, we performed a logistic regression analysis and reported the odds ratios and confidence intervals implemented in R.

We performed an explanatory analysis to determine the spatial distribution of the observed coccidian DNA positive proportion, and a variogram was plotted to assess the spatial autocorrelation by distance between the scats collected. Both analyses were implemented in ‘geoR’ and ‘PrevMap’ packages in R [56,57]. To model the spatial variation in coccidian DNA prevalence incorporating an unobserved spatial stochastic process, a geostatistical binomial logistic model using the Monte Carlo Maximum Likelihood method was implemented in the ‘PrevMap’ package [56]. The proposed risk
factors for *N. caninum* included in the model were density of cattle, relative abundance of wild canids and land use.

### 3.4 Results

#### 3.4.1 Wild canid relative abundance and host identification

The index of wild canid relative abundance ranged from 0 to 1.5 scats per km*day*, see Figure 3.3. Classification of the 285 scats by morphology showed that 78.9% scats were coyote, 17.2% fox and 3.9% other. To verify the visual identification of wild canid species for scats, we amplified the mtDNA in the feces. Seventy samples amplified out of a subset of 103 that were genetically analyzed. The genetic analysis of the 70 samples showed that 70% (49/70) scats were coyote and 30% (21/70) were red fox. Based on genetic analyses, the proportion of coyote and non-coyote scats in each certainty level were similar to expected proportions (*G*₂=1.334, *P* value= 0.61), see Table 3.1.
The index of wild canid relative abundance ranged from 0 to 1.5 scats per km*day (demonstrated by the size of the circle), by using the morphological classification. Coyote relative abundance (green) ranged 0 to 1.2 scats per km*day. Fox (purple) relative abundance ranged from 0 to 0.21 scats per km*day.

Figure 3.3 Relative abundance of wild canids at southeast Ohio.
Table 3.1 Host identification.

Observer certainty levels of coyote scats were compared to the actual coyote scats in the samples, which were determined by analysis of mtDNA (total $n = 57$). The expected number of coyote scats was calculated by multiplying the total number of scats in each certainty category by the certainty level. The expected proportion of coyote scats was calculated by dividing the number of expected of each certainty level by the total of expected. The non-coyote scats all were identified as red fox.

<table>
<thead>
<tr>
<th>Observer Certainty Level of Coyote Scats by mtDNA</th>
<th>No. Coyote Scats by mtDNA</th>
<th>No. Non-Coyote Scats by mtDNA</th>
<th>Observed Proportion of Coyote Scats</th>
<th>Expected No. Coyote Scats</th>
<th>Expected Proportion of Coyote Scats</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>30</td>
<td>14</td>
<td>0.73</td>
<td>44</td>
<td>0.80</td>
</tr>
<tr>
<td>90</td>
<td>7</td>
<td>1</td>
<td>0.17</td>
<td>7.2</td>
<td>0.13</td>
</tr>
<tr>
<td>70</td>
<td>4</td>
<td>1</td>
<td>0.097</td>
<td>3.5</td>
<td>0.07</td>
</tr>
</tbody>
</table>

G2=1.334, P. value= 0.61
3.4.2 Coccidian oocysts prevalence

Overall, fifty-one or 18% (95% CI: 13.8-22.7%) samples out of a total of two hundred eighty-five samples tested positive for the presence of coccidian DNA. Based on morphological identification of scat methods, from the 225 coyote scats analyzed only 19.6% demonstrated the presence of coccidian DNA whereas 49 of fox scats analyzed indicated 12.3%, but the difference in coccidian DNA prevalence between coyote and fox was not statistically significant ($X^2=0.99$, P. value= 0.32). The detection limit of the universal coccidian PCR assay was the equivalent of DNA-derived from 20 *N. caninum* tachyzoites and two *H. heydorni* oocysts (Sinnott et al., Unpublished results). The microscopic visualization test showed 125/285 being positive for *Neospora*-like oocysts from which 32 tested positive for both visual and PCR tests. The Cohen’s Kappa coefficient measuring agreement between the visualization and PCR determined slight agreement (0.049; 95% CI: 0.15-0.24).

*N. caninum* and *H. heydorni* specific PCR assays performed on the 51 coccidian DNA positive samples demonstrated very few positives. All samples were negative for *N. caninum* DNA and three coyote identified samples (2 genetically and 1 morphologically identified) (1.1%; 95% CI: 0.35%-3.0%) were positive for *H. heydorni* DNA. The *N. caninum*-specific assay was able to detect DNA from as few as the equivalent of two tachyzoites and did not amplify *H. heydorni* negative controls (Sinnott et al., Unpublished results). The *H. heydorni*-specific assay was able to detect DNA from two *H. heydorni* oocysts and did not amplify *N. caninum* negative controls (Sinnott et al., Unpublished results). We calculated an upper 95% confidence interval of *N. caninum*
DNA environmental prevalence of 7.2%, determining that a potential maximum of 160
1km² hexagons might have positive *N. caninum* DNA scats out of the 2223 total
hexagons in the study area.

Sequencing of a subset of the coccidian DNA positive sample amplicons, negative
for *N. caninum* and *H. heydorni* DNA, indicate the presence of *Sarcocystis*, *Eimeria*, and
*Cystoisospora* DNA.

3.4.3 Environmental exposure and scat freshness

The weather conditions to which the scats were exposed were as follows: temperature ranged from 16.4°C to 24.1°C, humidity from 67% to 77% and precipitation from 1.03 to 13.6 mm. We found that for a one degree Celsius increase in temperature, the odds of detecting coccidian DNA positive samples increased by a factor of 1.02 (95% CI: 1.01-1.04). Additionally, we found statistical significance in the odds of detecting coccidian DNA positive scats as freshness of the scats increased. Thus scats with a fresh value of one (most fresh) had an odds ratio of 2.43 (95% CI: 1.14-5.48) when compared with a fresh value of three (least fresh) and scats with a fresh value of two had an odds ratio of 1.04 (95% CI: 0.4-2.53) when compared with a fresh value of three.

3.4.4 Spatial distribution and risk factors association

The map of the observed positive proportion of coccidian DNA positive samples obtained from the explanatory analysis is presented in Figure 3.4. The variogram showed a very low scale of spatial correlation for the coccidian DNA prevalence, see Figure 3.5.
Figure 3.4 Distribution of the observed positive proportion of coccidian DNA in the study area.

The size of the circle demonstrates number of feces tested by transect (1 to 23). Positive samples ranged from 0 to 7 and negative ranged from 0 to 17 by transect.
In logistic regression models with and without spatially structured error, none of
the explanatory variables were statistically significant. Descriptively, from 73 scats
collected in areas with a “crop” land use, 17.8% tested positive for coccidian DNA; from
101 scats collected in “pasture” land use, 17.8% tested positive for coccidian DNA; and
from 111 scats collected in “wood” land use, 18.0% tested positive for coccidian DNA.
The index of density of cattle ranged from 0 to 1.4 cattle per acre. The geostatistical
binomial model parameters are shown in Table 3.2.
Figure 3.5 Empirical variogram for the logit transformation of the observed coccidian prevalence.

A low scale of spatial correlation is seen in this study.

Table 3.2 Mean and standard deviation for the geostatistical binomial model parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean and (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_0$</td>
<td>-1.679790 (0.060947)</td>
</tr>
<tr>
<td>$\sigma^2$ (variance of the Gaussian process)</td>
<td>0.11940 (1.9237)</td>
</tr>
<tr>
<td>$\phi^2$ (scale of the spatial correlation)</td>
<td>0.72887 (0.7892)</td>
</tr>
</tbody>
</table>
3.5 Discussion

The goal of this study was to explore the role of wild canids in the dynamics of infectious diseases that spill over to ruminant species, specifically *Neospora caninum*, in southeastern Ohio. As one of the most important top terrestrial predators in North America, the coyote is thought to play a role in the transmission of several infectious diseases at the wildlife-livestock interface. For example, coyotes are being used as sentinels to evaluate bovine tuberculosis in deer [58]. In the case of neosporosis, coyotes are a definitive host [22]; that is, they host the sexually mature organism and allow reproduction [59]. In contrast, red foxes have been suggested as an intermediate host rather than a definitive host for *N. caninum* by experimental and observational studies [60,61,62,63]. Unlike the coyote relative abundance in Ohio, the red fox relative abundance in Ohio has decrease threefold from 1990 to 2011 [26,64]. The estimated proportion of coyote scats (70%) and fox scats (30%) as determined by genetic analysis reflects these differences in relative abundance. Therefore, the coyote and red fox differences in the transmission cycle of *N. caninum* and differences in their relative abundance in Ohio suggest that continued changes in wild canid diversity might affect the observed epidemiology of the parasite in southeastern Ohio. Although we did not find a statistically significant difference between the proportions of coccidian DNA positive scats for coyote versus red fox, more thorough sampling of specific species of coccidia such as low-prevalence pathogens like *N. caninum* over time might reveal differences.

Our results show a potential maximum *N. caninum* environmental prevalence of 7.2% in the study area in southeastern Ohio. Yet, this results in a 0% prevalence estimate
for *N. caninum* in our study area during the sampling period. Similar to our results, other worldwide investigations based on canid fecal analysis have shown low prevalence [39,65,66,67,68,69], see Figure 3.6. The potential low prevalence estimated for the environmental phase of *N. caninum* shed by coyotes and foxes could be explained by 1) the shedding pattern and the time of collection; 2) the load of oocysts shed; 3) the age structure of the canid populations; 4) the type of prey or food source ingested; 5) the reliability of the laboratory tests used (i.e. microscopy, molecular analysis); 6) the environmental conditions to which the feces were exposed; and 7) a truly low infection rate of canids with *N. caninum* at endemic equilibrium. Our results seem to at least support hypotheses number 6 and number 7.
Figure 3.6 Low prevalence of *N. caninum* and 95% confidence intervals of diverse studies.

Confidence intervals were approximated for each study, accordingly to the ‘score’ method as implemented in ‘prevalence’ package in R (Devleesschauwer et al., 2014).
In experimentally infected dogs, shedding of oocysts has been suggested to be affected by the dog’s prior exposure to the parasite, and various shedding patterns and load of oocysts have been observed [10,70,71,72]. With respect to age, young coyotes showed resistance to experimental infection [8], while young dogs shed significantly more oocyst than adult dogs [72].

The diet of coyotes and red foxes varies depending on seasonal availability; rodents, birds, rabbits and fruit are the most common items in their diet. However, they feed also on white-tailed deer and on domestic ruminants [20,73]. Gondim et al., [40] experimentally showed that dogs fed with infected calf tissue shed more oocysts than dogs fed with infected mouse tissue. Moreover, Sobrino et al. [74] suggested that differences in *N. caninum* prevalence between red foxes and wolves in Spain might be due to the wolf’s diet being primarily based on ruminants, while the red fox is omnivorous. Therefore, *N. caninum* shedding or infection acquired by carnivores might vary depending on the intermediate host ingested [62,75,76]. The period during which we sampled was after deer hunting season in Ohio and corresponded to birthing of most species, including fawns [77]. Thus the effect of ingested deer-offal left in the field (‘prior exposure’) and deer placentas (‘current exposure’) might be a source of an environmental variation of oocyst prevalence, but inferences cannot be drawn from this study. A seasonal diet study in this region might provide knowledge between prey consumed and oocyst prevalence.

Discussions on identification and differentiation of *N. caninum* oocysts from other morphologically similar coccidian parasites have advanced diagnostic tools
[44,78,79,80]. Similar to others, our methodology of identification and differentiation of coccidian oocysts was based on microscopy followed by molecular analysis [68,69]. Comparable to our findings, Elmore et al. [81] also reported a low agreement between microscopy and molecular methods to identify small coccidian oocysts. Therefore, investigations that relied only on microscopic identification of small coccidian oocysts might underestimate or overestimate prevalence [33,39,60,68,69].

Information about the viability of *N. caninum* oocysts exposed to chemical and physical treatments have been assessed by Alves Neto et al. [82]. They found two treatments to be effective to environmentally inactivate *N. caninum* (a temperature of 100 °C for 1 min and 10% sodium hypochlorite for 1hr). Moreover, a study on the viability of *Toxoplasma gondii* oocysts in soil determined that damp conditions increased viability of the oocysts when compared with dry conditions [83]. Expanding the discussion on the environmental contamination and detectability of DNA of coccidian oocysts, our results showed that increasing temperature within the range of 16.4°C to 24.1°C increased the probability that a randomly selected scat will test positive for coccidian DNA. Thus a temperature threshold of survival or detectability might exist. Additionally, we found that detecting a coccidian DNA positive scat decreased as the feces aged, which aligns to coccidian oocyst literature [84,85]. However, neither precipitation nor humidity were statistically significant predictor variables. Also, an initial suspension of the scat with a detergent-based solution could have resulted in a better extraction of parasite ova from dry/older feces [84]. These environmental effects on recovery of oocysts might be used in future sampling plans and analyses to adjust observed prevalences.
Literature describes that canids are considered essential in the life cycle of *N. caninum*, yet detecting *N. caninum* oocysts from canids in natural settings has been challenged [76]. French et al., [9] suggested that low levels of *N. caninum* horizontally transmitted by canids (0.85-9.0 per 100 cow-years) could maintain a low cattle prevalence in the UK, and thus a bi-directional transmission could also be plausible. This could indicate that the issue is important even if the wild canid oocysts’ prevalence is low. Hence, the persistence of *N. caninum* in a herd might cause endemic abortions, thus causing an economic burden to the farmer [4]. Additionally, the resilience of this parasite could pose a conservation problem to non-domestic livestock.

The distribution of the prevalence of DNA of coccidian oocysts had a low scale of spatial correlation, and cattle density, wild canid’s relative abundance and land use did not explain its distribution. The many hosts and parasite species involved and the complex life cycles of coccidian parasites could explain the observed spatial distribution of the parasites. Thus, further studies on specific coccidian genera and increasing sample size of locations might be needed to elucidate mechanisms of their persistence in the environment and in the populations in southeastern Ohio. Because our study area harbors various species of ruminants and differed exposure could be expected related to herd management protocols, a combined empirical and theoretical approach to understanding the role of different ruminants (i.e. host related immunity) would add clarification in the dynamics of *N. caninum* at the community level.
3.6 Conclusions

To our knowledge, this study is the first to survey the environmental phase of *N. caninum* shed in wild canid scats in natural settings. We suggest a minor role of coyotes and red foxes in the disease dynamics of the environmental phase of *N. caninum* as we estimated a prevalence based on our sample results of 0%. Improving knowledge of the environmental contamination of *N. caninum* due to carnivores may help to improve ungulate reproduction by directing appropriate interventions for the multifaceted issues surrounding the wildlife-livestock interface. No existing scientific evidence, including our study, suggests that control of coyotes or foxes will control neosporosis in ungulates in our study area. Furthermore, adaptive and integrated interventions that lead to healthy predator populations may have other benefits for the health and welfare of wild populations, managed non-domestic populations, and the livestock industry. Finally, further epidemiological studies in natural settings might describe complex interactions among weather conditions and abundance and richness of hosts that lead to the transmission or lack thereof of infectious diseases at the wildlife-livestock interface.

3.7 Acknowledgements

We would like to thank the volunteer field assistants, farmers and *the Wilds* for granting research permission and collaboration. Also, we would like to thank Craig Hicks (USDA Wildlife Services, National Wildlife Disease Program) for providing advice on wildlife collection permits and providing control sample material. We are grateful for the great discussions provided by Stanley Gehrt, Laura Pomeroy, Hyeyoung Kim and the
Disease Ecology and Computer Modelling Laboratory group. We are very grateful for insight provided from the Spatial and Temporal Statistical Modelling for Population Health ISVEE 2015 workshop. C.A. Bremer at The Ohio State University College of Veterinary Medicine for her guidance regarding parasite visualization. Thanks to MCIC-OARDC and Jean Dubach at Loyola University Medical Center for molecular support on genetic analysis for host identification. This research has been financially supported by a gift from Duncan Alexander and grants from OSU CARES, Columbus Zoo & Aquarium and a summer research fellowship from NIH T35 OD010977.

3.8 References


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Chapter 4: Host Species Heterogeneity in the Epidemiology of *Neospora caninum*

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4.1 Abstract

The role of host species heterogeneity in the epidemiology of Neosporosis remains under-studied, although it is clear that a number of herbivore species are susceptible to *Neospora caninum* infection. Our goal was to describe host species heterogeneity in the epidemiology of *N. caninum* circulating in a community. We estimated immunological and transmission dynamics by comparing catalytic and reverse catalytic infectious disease models with age-structured and constant force of infection in three adjacent ruminant populations. Also, we estimated the species-specific contribution to the persistence of this pathogen in the community by calculating the reproductive number for each population. Finally, we calculated the critical proportion of vaccination coverage to prevent an outbreak. Results show that immunity in cattle and Pére David’s deer wanes over time, suggesting that boosting immunity with vaccines might be a venue to prevent infection within those populations. For white-tailed deer, immunity appears to be lifelong; thus, natural boosting of the immune system might be occurring. The reproductive number in cattle was below the threshold for an outbreak (*R_t < 1*), meaning that transmission cannot be maintained within a homogeneous population of cattle; thus, an outside source is needed to re-introduce the pathogen. Pére David’s deer and white-tailed deer populations should both be able to maintain continuous chains of transmission (*R_t > 1*). Therefore, control of outside sources, while useful for disease control in cattle, might not be effective in deer. Community-level investigations like this allow us to better evaluate heterogeneities in transmission processes that could ultimately guide targeted control.
Key-words: catalytic model, community, ruminant, heterogeneity, reservoirs, targeted control

4.2 Introduction

Multi-host pathogens are inherently flexible making them successful and scary pathogens [1]. For example, Cleaveland et al. reviewed literature on pathogens of humans and domestic animals and found that >61% of human pathogens, >77% of livestock pathogens and >90% of carnivore pathogens infected multiple species [2]. Yet, the number of studies investigating the heterogeneity of transmission of pathogens within and between species is low compared to studies focused in a unidirectional transmission event and single-host pathogens [1,3]. Differentiating reservoirs from maintenance populations for multi-host pathogens is a challenge when interventions towards preventing and controlling spillover events to targeted populations need to be implemented. Reservoirs as defined by Haydon et al., (2002), are epidemiologically connected populations (e.g. including maintenance and non-maintenance populations) or environments in which pathogens can be maintained and transmitted to targeted populations [4]. Maintenance populations, on the other hand, are defined as populations that exceed the critical community size [4]. The critical community size is the minimum size of a closed population in which a pathogen is not likely to fade out after a major epidemic [5]. Interventions to control and prevent disease, logically, will differ depending on how the reservoir is constituted and who the targeted population is. Thus,
understanding host species heterogeneity in the transmission dynamics of infectious diseases seems essential to differentiate reservoirs and maintenance populations for targeted control of multi-host pathogens circulating in a community [6]. For example, a study on rabies virus in carnivores in the Serengeti ecosystem shows that domestic dogs could act as the principal maintenance host species; thus, efforts to control rabies spillover to humans should be targeted to them, as opposed to wild canids [7]. Similarly, a review on the role of susceptible species of antelopes in the epidemiology of peste des petits ruminants discusses that sheep can be infected asymptomatically, and thus, be the source of infection to highly susceptible species, such as gazelles. With a reported morbidity rate of >50% and a case fatality rate of 100%, gazelles would be a poor choice for disease control interventions in this multi-host community [8].

In the case of *Neospora caninum*, a protozoan parasite capable of infecting a wide range of canid and ungulate species [9,10,11], relatively few studies have described the role of host species/breeds heterogeneity in the epidemiology and transmission of Neosporosis [12,13,14]. The life cycle of *N. caninum* involves three known stages. Oocysts are shed to the environment in feces by the definitive host (i.e. dogs and wild canids) and consumed by the intermediate host, which are primarily ruminants such as cattle and deer. Tachyzoites and bradyzoites develop in the intermediate host, causing damage to infected tissues and resulting in abortion, maternal infertility and other clinical signs [11]. The disease is important due to the substantial economic losses the parasite causes to the cattle industry, both nationally and internationally, mainly derived from premature culling, reduced milk yields and abortions [15,16]. *N. caninum* is estimated to
cost $843 million dollars annually in US dairy farms alone [15]. Prevention of *N. caninum* infection in cattle is based on reducing direct and indirect interactions between the definitive host (e.g. canids) and the intermediate host (e.g. ruminants) [17]. Also, because transplacental transmission (cow-calf) seems to be the primary source of maintaining the parasite within the herd as opposed to horizontal transmission (e.g. via colostrum and/or interactions with canids), maintaining a closed herd [17] and vaccines [18] have been suggested as control strategies to limit infection. However, at the moment, there is no vaccine or effective treatment for *N. caninum* on the market [19]. Moreover, to our knowledge, current control and prevention strategies are not based on actual studies of transmission across species. Furthermore, neither identification nor inclusion of other plausible hosts (e.g., rodents and birds) has been explored as part of a *N. caninum* reservoir. Therefore, while current targeted populations may be part of the reservoir, they may not necessarily be maintenance populations.

In southeastern Ohio, differences in true prevalence for intermediate (ruminant) host species suggest differences in the epidemiology of *N. caninum* for these adjacent populations [20]. In addition, an evaluation of the environmental phase of *N. caninum* shed in wild canid scats, suggested that this environmental phase in the transmission to ruminants is likely rare [21]. Thus, the next logical step is to evaluate host species heterogeneity in the epidemiology of *N. caninum* in the community.

In natural settings, interspecies variation in immunity, species-specific management, species-specific behaviors, and age structure of herds are intrinsically linked factors confounding investigations of host species heterogeneity in the transmission of
pathogens. However, infectious disease models, such as the catalytic and reverse catalytic models, can be used to determine species-specific heterogeneities by estimating key transmission parameters [22]. The force of infection, which is the per capita rate at which susceptible individuals acquired the infection, and the per capita rate at which natural seropositivity wanes over time, can identify heterogeneities in transmission and specify targets for disease control [23]. In addition, the force of infection (FoI) can be used to calculate the basic reproductive number ($R_0$), which is defined as the average number of secondary cases arising from an average primary case in an entirely susceptible population, and can inform the maximum reproductive potential for an infectious disease in a population, and thus the species-specific contribution of the persistence of a pathogen in a community [23,24].

Therefore, our goal was to better understand the role of host species heterogeneity in the epidemiology of *N. caninum* circulating in a community. We evaluated the role of immunodynamics and age-structured transmission in three intermediate (ruminant) hosts by estimating and comparing their FoI and the rate of waning immunity, assuming both, constant and age-structured FoI. Also, we estimated the species-specific contribution to the persistence of this pathogen in a community by calculating the reproductive number of each of the three ruminant host species.
4.3 Materials and Methods

4.3.1 Study area and population

The ecological system is located in southeastern Ohio and contains our focal species: cattle (*Bos taurus*), Père David’s deer (*Elaphurus davidianus*) and white-tailed deer (*Odocoileus virginianus*). This region is rural and includes the largest conservation center in North America, the International Center for the Preservation of Wild animals (DBA, *the Wilds*). Several rare and endangered ruminant species are bred at *the Wilds* as part of their conservation program. Beef cattle farms predominate in the area surrounding the conservation center, but dairy cattle and small ruminant farms also are found in the community. White-tailed deer in this community are free-ranging wildlife capable of crossing fences and comingling with both domestic and non-domestic captive herds. This region allows us to explore mechanisms of the persistence of multi-host pathogens, such *N. caninum*, across the wildlife-livestock interface in a community of adjacent and intermingling populations.

4.3.2 Sample collection

In a cross-sectional study design, we collected jugular vein blood samples (10 ml per individual) during March 2013 for 37 (23 females, 13 males, 1 unrecorded) Père David’s deer managed at *the Wilds*. Birth date records were accessed from *the Wilds* database; thus, individuals’ age was calculated from the birth date to the bleeding date. For 137 female cattle, tail vein blood samples (10 ml per individual) were collected at the Muskingum Livestock Auction, from June 2014 to June 2015 (see below cattle selection).
In addition, we recorded breed, reproductive status, gestational age and cattle age. Reproductive status and gestational-fetal age [25] were evaluated through rectal palpation by the auction veterinarian and cattle age was determined through dentition [26] by an experienced worker at the auction. The same veterinarian and worker evaluated all cattle during the entire study collection. Forty-two samples (37 females and 5 males) from free-ranging white-tailed deer were obtained from the study area during Ohio’s hunting season in 2012, 2013 and 2014. Deer hunting season in Ohio extends from October to February. We collected 10ml of blood per deer directly from the heart, soon after death to avoid degradation of antibodies. Also, we collected brain, placenta and fetal tissues. We estimated and recorded individuals’ ages through dentition [27], reproductive status was recorded based on visual inspection of the uterus, and gestational-fetal age was estimated through crown-rump length measurements [28].

Cattle selection at the auction was conducted systematically and was stratified based on township, and weighted based on approximate numbers of cattle farms in each township using estimates provided by the four county (Muskingum, Morgan, Guernsey and Noble) extension agents in the area (supplementary material Table 1) . We continued to sample cattle arriving at the auction from the targeted townships until the sample size goal for that township was achieved. Additionally we limited our sampling to a maximum of three cows selected per owner/seller per auction day. Targeted townships were those that were located within 25 km radius from the Wilds. The area selected in this study (25 km radius) was based on the home range of coyotes, average 17.5 km² and follows previous study design [21]. The auction was used for sampling because adequate
management facilities do not exist on most farms in this area to effectively restrain cattle for blood collection. The cattle at auction are not a random sample of all cattle in the area – cattle at auction tend to be young healthy beef cattle and culled breeding/dairy stock. We selected only female cull cattle.

4.3.3 Laboratory testing

Serum was separated by centrifugation and collected tissues were frozen. Both, serum and tissues were stored at -20°C. All species’ serum was tested for *N. caninum* antibodies using a commercial cELISA kit (VMRD®, Inc., Pullman, WA, USA) and white-tailed deer tissues were tested for *N. caninum* DNA by using species-specific primers: Np4-Np7 targeting the *pNC-5* gene of *N. caninum* through PCR [29,30] at the Ohio Department of Agriculture Animal Disease Diagnostic Laboratory, Reynoldsburg, OH. The cELISA was performed according to manufacturer recommendations. Individual results were reported as percentage inhibition values; a value ≥ 30% inhibition was considered a positive result and <30% a negative result, as that cut-off is currently used by the manufacturer and has been validated for cattle [31]. For each white-tailed deer, tissue was tested in three pools (each 2-3 grams): 1) pool of brain (frontal lobe, cerebellum, cerebral cortex, and spinal cord), 2) pool of samples from placenta, cotyledon and uterus, multiple areas of each organ, and 3) pool of fetal tissue (brain, lung, heart, kidney, and liver).
4.3.4 Data analysis

Descriptive statistics were performed on breed, reproductive status, and gestational-fetal age for cattle, and reproductive status and gestational-fetal age for white-tailed deer. Age distribution of each species was also described. In addition, a logistic regression analysis was performed in R using the ‘stats’ package to estimate associations between sero-positivity and the potential confounders described above.

4.3.5 Models implementation, selection, and parameterization

To evaluate species-specific immunodynamics and age-structure of transmission, we used the true proportion of sero-positive individuals and age data. We calculated age-specific true prevalence (TP) by using Bayesian inference methods with informative prior distributions explicitly described in Moreno-Torres et al. (in press) [20]. We compared catalytic and reverse catalytic models with constant and age-structured FoI following the method of Pomeroy et al. [32], to determine which fit the true proportion of sero-positive individuals and age data better for each species. The catalytic model assumes lifelong immunity, while the reverse catalytic relaxes this assumption allowing to evaluate waning immunity [32]. Because Père David’s deer and white-tailed deer samples included 0.5 and 1 year old individuals, we took into account the possibility of maternal antibodies. For our purpose, we called maternal antibodies, antibodies found in less than 2 year old individuals. Those antibodies could be maternal antibodies or fetal antibodies from congenital infection with *N. caninum*. However, for cattle, we ignored any maternally derived antibodies because sampled individuals were at least two year of age, beyond the
lifespan of maternal antibodies. To select the model that best fit the data for each species, we used the Akaike’s Information Criterion (AIC) [33]. Therefore, we can assess which immunological and transmission process best described each studied population.

In the catalytic model, we assumed, first, a constant change of FoI per unit time (years). Then, we compared this with a model including annual changes in the FoI by fitting the data as a b-spline that varied by year with two spaced internal knots. Mathematically, let $\lambda(t)$ represent the age-structured FoI, let $a$ represent the age of each individual in years and let $t$ represent a unit of time in years. Accordingly to the catalytic model [22,32], assuming lifelong immunity, the age-specific seroprevalence considering maternal immunity is given by

$$P(a) = 1 - e^{-\int_{a}^{t} \lambda(t)da}$$

(1)

and the reverse catalytic model, assuming immunity last for an average duration of $1/\omega$ and then wanes [22], the age-specific seroprevalence considering maternal immunity is given by,

$$P(a) = \frac{\lambda(t)}{\lambda(t)+\omega} (1 - e^{\int_{a}^{t} (\lambda(t)+\omega)da}),$$

(2)
We estimated the FoI and the rate of waning immunity by maximizing the binomial likelihood of seropositive animals. Likelihood estimations were performed using the “optim” function in R.

We calculated the reproductive number ($R_t$), the number of secondary cases produced by a primary infectious case, by multiplying the estimated FoI and the average life expectancy of each species. Finally, we estimated the critical proportion of vaccination coverage, assuming a fully efficacious vaccine, required to prevent an outbreak as a $P_c=1-(1/R_t)$.

4.3.6 Ethics statement

Animal use protocols were reviewed and approved by the Ohio State University Institutional Animal Care and Use Committee (Protocol Number: 2012A00000154). Both captive species (cattle and Père David’s deer) were physically restrained in a chute and, once restrained, the procedure lasted about 10-15 minutes per animal. Individuals were sampled early in the morning to avoid heat stress and acutely stressed individuals were removed from the study. A white-tailed deer scientific collection permit was granted by the Division of Wildlife, Ohio Department of Natural Resources (Permit Number: 14-266). The collection of white-tailed deer samples was opportunistic during Ohio’s hunting season, thus the accessibility and availability of sample collection was restricted to the number of hunters that verbally consented to provide samples and their bag limits. The Wilds and the Muskingum Livestock Auction gave written consent to participate in this study. Both facilities provided specialized personnel to handle animals and the
necessary equipment to physically restrain animals. Cattle owners/sellers verbally consent cattle sampling at the auction and provided the township location where the cattle resided prior to the auction.

4.4 Results

Descriptively, the samples of the three ruminant’s populations varied in their age distributions, reproductive status and gestational age (Figure 4.1-4.5). For the 137 sampled cattle, 10% were dairy breeds, 25% crossbreed and 65% beef breeds (Appendix A, Table 4.4). In logistic regression models, there was no association between sero-positivity and breed, reproductive status, or gestational-age of cattle and white-tailed deer. As reproductive data were not available for the Père David’s deer, associations were not tested in this species. However, a previous retrospective study on *N. caninum* seroprevalence in the Père David’s deer herd found no correlation between calving rates and seropositivity [34].
Figure 4.1 Age distribution and reproductive status of the 137 sampled female cattle.

A total of 59% of cattle were open and 41% pregnant.
Figure 4.2 Gestational age of pregnant cattle.

Pregnant cattle were distributed in all three trimesters of gestation, indicated by the vertical red lines.
Figure 4.3 Age distribution of 37 (23 females, 13 males, 1 unrecorded) sampled Père David’s deer.

There were no individuals from 9 to 13 year old. Reproductive status was not recorded but pregnant individuals were in their third trimester of gestation, by The Wilds records and based on seasonality of breeding in this species.
Figure 4.4 Age distribution and reproductive status of 37 sampled female white-tailed deer.

There were 5 males sampled: 0.5 year old (n=1), 1.5 year old (n=3) and 2.5 year old (n=1) (not displayed in this figure). A total of 11% of white-tailed deer were open and 89% pregnant.
Gestational age of pregnant white-tailed deer.

Pregnant white-tailed deer were distributed in the second trimesters of gestation.

All white-tailed deer tissue samples were negative for *N. caninum* DNA by PCR test.

The proportion of sero-positive individuals per age by species is shown in Figure 4.6. The mode of the overall cattle population prevalence was 7.2% (95 PI% 3.9-12%), for Père David’s deer population prevalence was 53% (95 PI% 39.4-66%) and for white-tailed deer population prevalence was 53.5% (95 PI% 36-78%).
Figure 4.6 Apparent and true prevalence of proportion positive individuals by species and age.
Data used in models are presented in Table 4.1. The AIC values for the compared models can be seen in Appendix A, Table 4.6-4.8. For cattle, Père David’s deer and white-tailed deer the best-fit models were reverse catalytic, reverse catalytic with maternal antibodies and catalytic with maternal antibodies, respectively (Figure 4.7-4.9). The models with age-structured transmission were rejected for all of the populations. Parameter estimates varied depending on the type of data used, apparent versus true prevalence. Also, epidemiology differed among species as measured by differences in FoI, the reproductive number and immunodynamics. Based on the estimates of $R_t$, the two deer species seem to maintain chains of transmission within their populations, while cattle seem to need an outside source to maintain the pathogen within the population (Table 4.2).
Table 4.1 Number of samples positive using apparent and true prevalence, by species and age.

<table>
<thead>
<tr>
<th>Species</th>
<th>Age (years)</th>
<th>Apparent Prevalence</th>
<th>True Prevalence</th>
<th>Total sampled</th>
</tr>
</thead>
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<td><strong>Cattle</strong></td>
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<td>0</td>
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<tr>
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<td>18</td>
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<td></td>
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</tr>
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</tr>
<tr>
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<td>0</td>
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<tr>
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<td>16</td>
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<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
Figure 4.7 Model selection for cattle.

Reverse catalytic model was the best-fit model for cattle. Size of points represent the relative number of animals sampled with age.
Figure 4.8 Model selection for Père David’s deer.

Reverse catalytic model with maternal antibodies was the best-fit model for Père David’s deer. Size of points represent the relative number of animals sampled with age.
Figure 4.9 Model selection for white-tailed deer.

Catalytic model with maternal antibodies was the best-fit model for white-tailed deer.

Size of points represent the relative number of animals sampled with age.
Table 4.2 Parameter estimates from best-fit model with apparent (AP) versus true prevalence (TP)

<table>
<thead>
<tr>
<th>Species</th>
<th>Data</th>
<th>Model selected</th>
<th>Force of infection (years(^{-1}))</th>
<th>Duration of natural sero-positivity (years)</th>
<th>Maternal sero-positivity duration (years)</th>
<th>Reproduction number</th>
<th>Critical proportion to vaccinate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cattle</strong></td>
<td>AP</td>
<td>Reverse Catalytic</td>
<td>0.051</td>
<td>2</td>
<td>n/a</td>
<td>0.61</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>TP</td>
<td>Reverse Catalytic</td>
<td>0.055</td>
<td>2</td>
<td>n/a</td>
<td>0.66</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Pere David’s deer</strong></td>
<td>AP</td>
<td>Reverse Catalytic</td>
<td>0.55</td>
<td>4</td>
<td>1.5</td>
<td>8.85</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>TP</td>
<td>Reverse Catalytic</td>
<td>0.41</td>
<td>1</td>
<td>1.5</td>
<td>6.53</td>
<td>0.85</td>
</tr>
<tr>
<td><strong>White-tailed deer</strong></td>
<td>AP</td>
<td>Catalytic *Mat</td>
<td>0.135</td>
<td>lifelong</td>
<td>1.5</td>
<td>0.88</td>
<td>n/a</td>
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<tr>
<td></td>
<td>TP</td>
<td>Catalytic *Mat</td>
<td>0.170</td>
<td>lifelong</td>
<td>1.5</td>
<td>1.11</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*Mat is defined as the antibodies found in less than 2 year old individuals. Those antibodies could be maternal antibodies or fetal antibodies from congenital infection with *N. caninum.*
4.5 Discussion

Our goal was to describe host species heterogeneity in the epidemiology of a multi-host pathogen, *N. caninum*, circulating in a community. As in many natural settings, our study area supports diverse domestic and non-domestic species. This community structure is rarely investigated as an epidemiological continuum where each species might affect pathogen transmission or lack thereof. Study designs to obtain detailed data to measure transmission heterogeneities longitudinally in the field could be prohibitively expensive in labor and time. However, we have demonstrated that the most commonly and easily gathered data, serology and age, can be used to test hypotheses regarding transmission heterogeneities using mathematical models [22,32,35,36].

Our results show that immunity in cattle and Père David’s deer wanes over time, suggesting that boosting immunity with vaccines might prevent re-infection within those populations. For white-tailed deer, immunity appears to be lifelong; thus, natural boosting of the immune system might be occurring or natural infections may truly result in lifelong immunity to re-infection. In this case, vaccination would not necessarily add benefits to this species, but perhaps decrease horizontal transmission to definitive hosts, by preventing the formation of tissue cysts.

*N. caninum* is an obligatory intracellular parasite that seems to avoid host immunity by differentiating from tachyzoites into bradyzoites within cysts (tissue cysts). That differentiation changes the health status of a host from being acutely infected to chronically. Chronically infected individuals appear to be asymptomatic. However, during pregnancy, recrudescence (bradyzoites can transform back to tachyzoites) is
plausible, hence resulting in symptomatic individuals. Pregnancy causes changes in the immune system in order to accept the fetus, this disruption in the immune system allows the parasite to infect the fetus. Therefore candidate vaccines should prevent tachyzoite proliferation and dissemination to avoid transplacental transmission, and prevent tissue cyst formation [37]. Although various vaccine strategies, including live attenuated vaccines, killed parasite lysates, total antigens or antigen fractions from killed parasites, and subunit vaccines, have been investigated to mediate protection in mice and cattle models, there are still disadvantageous factors such as cost of production, safety and stability that limit their application [18].

The reproductive number for cattle was below the threshold for epidemic transmission ($R_t = 0.66$), suggesting that transmission cannot be maintained within cattle; thus, population heterogeneity or an outside source is needed to re-introduce the pathogen. This finding suggests that, for cattle, controlling outside sources of infection may be helpful in controlling disease.

Previously, French et al., 1999 studied transmission and control options of *N. caninum* in cattle. The authors implemented mathematical models in which vertical transmission and horizontal transmission inside the herd (shared colostrum) and from an outside source (canids) were evaluated in a dairy production system. Control strategies were assessed by altering birth and death rates and horizontal transmission patterns. As a result, they found that annual culling and not breeding replacement heifers from infected cattle were the most successful control measures in a dairy cattle farm. One of their remarkable findings was that, even with a high vertical transmission probability, some
horizontal transmission was needed to maintain the endemism of the parasite in a herd [38]. A finding that is supported by our results.

Unlike cattle, both Père David’s deer and white-tailed deer could maintain continuous chains of transmission ($R_t > 1$) within their populations. Therefore, control of outside sources would not likely affect disease transmission in deer.

Seroprevalence studies in cattle, water buffalo (*Bubalus bubalis*) and Père David’s’ deer have suggested both age-specific and constant *N. caninum* prevalence with age [34,39,40,41,42]. In our study none of the three ruminant populations showed age-structured FoI. This suggests, accordingly to Muench 1959, that vertical transmission is the main route of transmission with low rates of horizontal transmission. Previous work in our study area found a low environmental prevalence of *N. caninum* oocysts shed by wild canid hosts, which supports the idea that transmission from canids to ruminants is infrequent (Moreno-Torres, in review). Although differences in the magnitude of the FoI per species captured species-specific differences in disease transmission, the FoI cannot distinguish individual mechanisms that affect the health status of the individual, such as, changes in susceptibility during gestation and specific environmental exposure (e.g., herd management). Additionally, the rates estimated, such as $R_t$, will vary depending on the studied populations, as herd management differences, contact structure and ecological interactions could modify transmission [23].

Differences in *N. caninum* seroprevalence, rates of abortion and immune responses between cattle breeds have been suggested [13,14,43,44]. A proportion of each of the three ruminant populations sampled were in various stages of gestation. However, neither
breeds, reproductive status nor gestational age were associated with the probability of being seropositive. Also, none of the white-tailed deer tissue that was tested by PCR was positive; thus, seropositive individuals were either exposed but not infected at sampling or detection was limited due to a low load of parasites in the tissues of infected white-tailed deer. This finding could also support that white-tailed deer immunity is lifelong.

Differentiating reservoirs from maintenance populations in the epidemiological continuum is one of the goals to target control and prevention for domestic and wildlife populations. The methods we have demonstrated suggest which populations maintain disease (deer) and which require outside introduction of the pathogen (cattle). In addition, these methods guide targeted control and prevention by suggesting in which species biosecurity will be helpful (cattle) or vaccination would be helpful (cattle and Père David deer). To better confirm our findings, a further evaluation that incorporates a larger sample size for white-tailed deer and cattle is suggested. Finally, to our knowledge, this is the first study to suggest control and prevention strategies based on a better understanding of the heterogeneity of transmission across species.

4.6 Supporting Information

Supplementary file for sample size calculation of cattle (Table 4.3, 4.4), descriptive statistics of cattle breeds (Table 4.5), and AIC values of all 16 contrast models with apparent and true prevalence data (Table 4.6-4.8).
4.7 Acknowledgments

We would like to thank the hunters, farmers, volunteer field assistants, the Wilds and Muskingum Livestock Auction for granting research permission and collaboration. In addition, we would like to thank Craig Hicks (USDA Wildlife Services, National Wildlife Disease Program) for providing advice on wildlife collection permits, Dr. Foster Anderson (Auction veterinarian) and Randi (Auction worker) for providing expertise on handling and sampling collection, and Pallavi Oruganti and Erika Helgeson for field and laboratory assistance. Also, we would like to thank the Ohio Department of Agriculture Animal Disease Diagnostic Laboratory, Reynoldsburg, OH for performing the serological and molecular tests. We are grateful for the insightful discussions provided by the Disease Ecology and Computer Modelling Laboratory group.

4.8 References


4.9 Supplementary file

Table 4.3 Sample size for cattle.

<table>
<thead>
<tr>
<th>Total Population (beef livestock)</th>
<th>Expected frequency</th>
<th>Confidence limits %</th>
<th>Confidence level %</th>
<th>Total sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>19150</td>
<td>24</td>
<td>6</td>
<td>95</td>
<td>199</td>
</tr>
</tbody>
</table>

The sample size calculation was done by Epi Info 7. The beef livestock population estimate comes from the sum of the number of farms at the township level multiplied by 50 (the estimated number of individuals per farm). Those farm estimates were given by OSU extension personnel at Muskingum, Morgan, Noble and Guernsey counties. We are using 24% of prevalence reported at ODA/ADDL with a 6% of precision (18-30%), 95% Confidence intervals. Therefore we are requesting to sample up to 199 cattle at the Auction. The number of individuals to sample by township at the auction was calculated by weighting the total sample by the livestock population size at the township level.
Table 4.4 Stratified sample size of cattle by township

<table>
<thead>
<tr>
<th>Number</th>
<th>County</th>
<th>Township</th>
<th>No. farms</th>
<th>No. cattle</th>
<th>Sample Size</th>
<th>Sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Guernsey</td>
<td>Jackson</td>
<td>10</td>
<td>500</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Guernsey</td>
<td>Richland</td>
<td>12</td>
<td>600</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
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<td>Guernsey</td>
<td>Spencer</td>
<td>15</td>
<td>750</td>
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</tr>
<tr>
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</tr>
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<td>Wayne</td>
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<td>50</td>
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Table 4.5 Breeds of cattle sampled.

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<td>Charolais</td>
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<td>Hereford</td>
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</tr>
<tr>
<td>Shorthorn</td>
<td>7</td>
</tr>
<tr>
<td>Simmental</td>
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</tr>
<tr>
<td><strong>Dairy</strong></td>
<td>14</td>
</tr>
<tr>
<td>Brown Swiss</td>
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</tr>
<tr>
<td>Guernsey</td>
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</tr>
<tr>
<td>Holstein</td>
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</tr>
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</tr>
<tr>
<td>Angus and Hereford</td>
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</tr>
<tr>
<td>Angus and Simmental</td>
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</tr>
<tr>
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</tr>
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<tr>
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<td>Simmental cross</td>
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<tr>
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Table 4.6 AIC values of the cattle models.

<table>
<thead>
<tr>
<th>Species</th>
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<th>Model</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>AP</td>
<td>Catalytic</td>
<td>31.0162</td>
</tr>
<tr>
<td></td>
<td>AP</td>
<td>Reverse Catalytic</td>
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</tr>
<tr>
<td></td>
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</tr>
<tr>
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<td>AP</td>
<td>Reverse Catalytic Mat</td>
<td>31.5784</td>
</tr>
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<td></td>
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<td>Catalytic Age</td>
<td>38.2939</td>
</tr>
<tr>
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<td>AP</td>
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<td>34.8141</td>
</tr>
<tr>
<td></td>
<td>AP</td>
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</tr>
<tr>
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<td>AP</td>
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<td>Catalytic</td>
<td>29.7305</td>
</tr>
<tr>
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<td>TP</td>
<td>Reverse Catalytic</td>
<td>28.1471</td>
</tr>
<tr>
<td></td>
<td>TP</td>
<td>Catalytic Mat</td>
<td>32.0387</td>
</tr>
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<td>Reverse Catalytic Mat</td>
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</tr>
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<td>Catalytic Age</td>
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<td>40.0188</td>
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Table 4.7 AIC values of the Père David’s deer models.

<table>
<thead>
<tr>
<th>Species</th>
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<th>Model</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Père David’s deer</em></td>
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<td>Catalytic</td>
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<td>Reverse Catalytic</td>
<td>32.0546</td>
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<td></td>
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<td>Catalytic Mat</td>
<td>35.8637</td>
</tr>
<tr>
<td></td>
<td>AP</td>
<td>Reverse Catalytic Mat</td>
<td>31.2982</td>
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<td></td>
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<td>Catalytic Age</td>
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<td></td>
<td>AP</td>
<td>Reverse Catalytic Age</td>
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<tr>
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<td>Reverse Catalytic Mat</td>
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</tr>
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<td>Catalytic</td>
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</tr>
<tr>
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<td>Reverse Catalytic</td>
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<td>Catalytic Mat</td>
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<td>TP</td>
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</tr>
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<td>Catalytic Mat Age</td>
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Table 4.8 AIC values of the white-tailed deer models.

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<th>Species</th>
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</thead>
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<td>Catalytic</td>
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</table>
Chapter 5 : Concluding remark

Studies on multi-host pathogen systems have slowly increased in comparison to single-host systems. In addition, recognition of the emergence and re-emergence of pathogens across species has promoted concepts such as the One Health. The One Health concept integrates knowledge of various disciplines with the goal of better understanding health-related complexities such as pathogen transmission and persistence at various spatial and temporal scales. We combined observational and modeling methods, which resulted in a better understanding of the wildlife-livestock interface of *N. caninum*. First, we estimated the true prevalence of American bison, Père David’s deer and white-tailed deer species by using Bayesian inference. Bayesian methods could be used to overcome the constraints on validating tests in wildlife species. We advise that surveillance programs need to take into account that some infected white-tailed deer may not test positive. We identified differences between apparent and true prevalence (TP). Differences in TP for some species suggest differences in the epidemiology of *N. caninum* for these co-located populations. Second, we evaluated the environmental phase of *N. caninum* shed in wild canid scats in natural settings. Results suggested that the role of this environmental phase in the transmission to ruminants is likely minor. Also, by incorporating weather conditions, relative abundance of wild canids and density of
ruminants a holistic description and understanding of the *N. caninum* life cycle was possible. Finally, we evaluated the role of host species heterogeneity in the epidemiology of *N. caninum* circulating in a community. Differentiating reservoirs from maintenance populations in the epidemiological continuum is one of the goals to target control and prevention to either domestic or wildlife populations. We identified differences in the role of immunity, age structure, and maternal and/or fetal antibody duration in cattle, white-tailed deer and Père David’s deer. Also, we estimated the species-specific contribution to the persistence of this pathogen in a community. Our results suggest which populations maintain disease (deer) and which require outside introduction of the pathogen (cattle). In addition, these results suggest in which species biosecurity will be helpful (cattle) or vaccination would be helpful (cattle and Père David deer). In conclusion each chapter fills a specific gap in the understanding of *N. caninum* and overall this dissertation shows an approach for exploring the complexity of multi-host pathogens. Future multi-disciplinary work building on the foundation I have created, could explore human dimensions, temporal and spatial pathogen patterns, and species-specific effects on multi-host pathogen persistence.
Chapter 6 Bibliography

Chapter 1


Chapter 2


Chapter 3


33. Gupta S, Wolfe B, Rajala-Schultz P. Identification of Neospora caninum oocysts from feces of Ohio coyotes (Canis latrans) using quantitative PCR. ; 2011; Columbus. The Ohio State University.

Chapter 4


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